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(71) Applicant *(for all designated States except US)*: **SMITHKLINE BEECHAM CORPORATION** [US/US]; One Franklin Plaza, Philadelphia, PA 19103 (US).

(71) Applicant *(for KP, KR only)*: **LG CHEMICAL LTD.** [KR/KR]; 104-1, Moonji-dong, Yusong-gu, Taejon, 305-308 (KR).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **APPELBAUM, Peter, C.** [US/US]; 500 University Drive, Hershey, PA 17033 (US). **BAST, Darren** [CA/CA]; 711 Concession Street, Hamilton, Ontario L8V 1C3 (CA). **CITRON, Diane, M.** [US/US]; 2620 Arizona Avenue, Santa Monica, CA 90404 (US). **CRABB, Donna, M.** [US/US]; 845 19th Street, Birmingham, AL 35294 (US). **CREDITO, Kim, L.** [US/US]; 500 University Drive, Hershey, PA 17033 (US). **DAVIDSON, Ross, J.** [CA/CA]; 5788 University Avenue, Halifax, Nova Scotia B3H 2V8 (CA). **DAVIES, Todd** [US/US]; 500 University Drive, Hershey, PA 17033 (US). **DEAZAVEDO, Joyce** [CA/CA]; 600 University Avenue, Toronto, Ontario M5G 1XG (CA). **DUBOIS, Jacques** [CA/CA]; 811 Place Le Chateaumont, Fleurimont, G1J 4W2 (CA). **DUFFY, Lynn, B.** [US/US]; 845 19th Street, Birmingham, AL 35294 (US). **DUNCAN, Carla** [CA/CA]; 1280 Main Street W., Hamilton, Ontario L8S 4L8 (CA). **EDNIE, Lois, M.** [US/US]; 500 University Drive, Hershey, PA 17033 (US). **GOLDSTEIN, Ellie, J., C.** [US/US]; 2021 Santa Monica Bvld., Santa Monica, CA 90404 (US). **HOELLMAN, Diane, B.** [US/US]; 500 University Drive, Hershey, PA 17033 (US). **KELLY, Linda, M.** [US/US]; 500 University Drive, Hershey, PA 17033 (US). **LOW, Donald, E.** [CA/CA]; 600 University Avenue, Toronto, Ontario M5G 1XG (CA). **PANKUCH, Glenn, A.** [US/US]; 500 University Drive, Hershey, PA 17033 (US). **SEARCEY, Karen, B.** [US/US]; 845 19th Street, Birmingham, AL 35294 (US). **ST-PIERRE, Claude** [CA/CA]; 980 Laliberte South, St-Elie d'Orford, J0B 2S0 (CA).

(74) Agents: **GIMMI, Edward, R.** et al.; SmithKline Beecham Corporation, Corporate Intellectual Property, UW2220, 709 Swedeland Road, P.O. Box 1539, King of Prussia, PA 19406-0939 (US).

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(54) Title: METHODS OF USE OF FLUOROQUINOLONE COMPOUNDS AGAINST BACTERIA

(57) Abstract: This invention relates, in part, to newly identified methods of using quinolone antibiotics, particularly a genifloxacin compound against certain bacteria.

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METHODS OF USE OF FLUOROQUINOLONE COMPOUNDS AGAINST BACTERIA

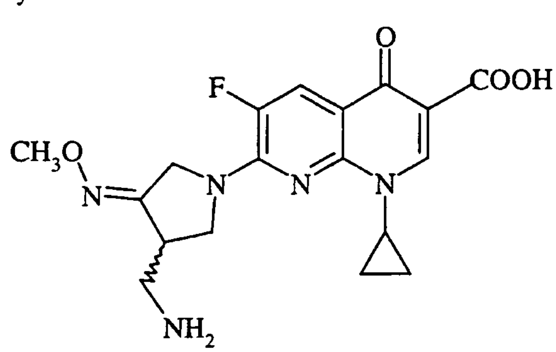
FIELD OF THE INVENTION

This invention relates, in part, to newly identified methods of using quinolone antibacterials, particularly a gemifloxacin compound against pathogenic bacteria, including maxillary sinus pathogenic pathogenic bacteria, such as penicillin-resistant and ciprofloxacin-resistant bacteria, especially resistant *Streptococcus pneumoniae,* anaerobic bacteria, especially unusual anaerobic bacteria, atypical upper respiratory pathogenic bacteria, Mycoplasma bacteria, such as *Mycoplasma pneumoniae,* pneumococcal bacteria, such as *Streptococcus pneumoniae,* fluoroquinolone resistant *S. pneumoniae*, especially ciprofloxacin or trovafloxacin resistant *S. pneumoniae,* quinolone-resistant bacteria, such as quinolone-resitant pneumococcal bacteria, certain *Haemophilus influenzae* strains, especially rare *Haemophilus influenzae* strains, ciprofloxacin-sensitive, ciprofloxacin-resistant bacteria and trovofloxacin-resistant bacteria, such as pneumococci, especially ciprofloxacin-sensitive and ciprofloxacin-resistant *Streptococcus pneumoniae.*

BACKGROUND OF THE INVENTION

Quinolones have been shown to be effective to varying degrees against a range of bacterial pathogens. However, as diseases caused by these pathogens are on the rise, there exists a need for antimicrobial compounds that are more potent than the present group of quinolones.

Gemifloxacin mesylate (SB-265805) is a novel fluoroquinolone useful as a potent antibacterial agent. Gemifloxacin compounds are described in detail in patent application PCT/KR98/00051 published as WO 98/42705. Patent application EP 688772 discloses novel quinoline(naphthyridine)carboxylic acid derivatives, including anhydrous (R,S)-7-(3-aminomethyl-4-methoxyiminopyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid of formula I.



I

PCT/KR98/00051 discloses (R,S)-7-(3-aminomethyl-4-*syn*-methoxyimino-pyrrolidin-1-yl)-1-cyclopropyl-6-fluoro-4-oxo-1,4-dihydro-1,8-naphthyridine-3-carboxylic acid methanesulfonate and hydrates thereof including the sesquihydrate.

Provided herein is an invention based, in part, on a number of significant discoveries made using a gemifloxacin compound against a range of bacteria.

One such invention made using a gemifloxacin compound against a range of respiratory pathogens, demonstrated that the activity of a gemifloxacin compound used was superior to a number of quinolones, as described in more detail herein. Gemifloxacin compounds are valuable compounds for the treatment of acute or chronic sinusitis caused by a range of respiratory pathogens, including those resistant to usual oral therapy, thereby filling an unmet medical need.

While *in vitro* testing of new antimicrobial compounds is often extensive, these studies tend to focus on a limited range of typical anaerobic bacterial pathogens (Cormicon, et al., *Antimicrob. Agents Chemother.*, 41:204-211, 1997; Hohl, et al., *Clin. Microbiol. Infect.*, 4:280-284, 1998; Marco, et al., *J. Antimicrob. Chemother.*, 40:605-607, 1997). Moreover, the activity of gemifloxacin against typical anaerobic bacteria has been reported (Goldstein, et al., *Antimicrob. Agents Chemother.*, Submitted); it showed activity against *Bacteroides fragilis* and certain *Prevotella* and *Porphyromonas* strains, but only limited activity against *B. thetaiotaomicron*, *B. distasonis* and *B. ovatus*. Data is lacking about the activities of new quinolone compounds against many of the less frequently encountered anaerobic pathogens, such as *Actinomyces* spp., *Anaerobiospirrilum* spp., *Porphyromonas* spp., and *Bilophila wadsworthia*.

Provided herein is an invention based, in part, on a significant discovery made using a gemifloxacin compound against various unusual, though medically important, anaerobic bacteria, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein. Gemifloxacin compounds are valuable compounds for the treatment of bacterial infections caused by a range of anaerobic pathogens, including those resistant to usual oral therapy, thereby filling another unmet medical need.

Further provided herein is an invention based, in part, on a significant discovery made using a gemifloxacin compound against a range of variety of *Legionella* isolated from nosocomial or acquired respiratory tract infections and from environmental sources, demonstrating the activity of the gemifloxacin compound used was superior to a number of

quinolones as described in more detail herein. Gemifloxacin compounds are valuable compounds for the treatment of diseases caused by or related to atypical respiratory tract pathogens, thereby filling still another unmet medical need.

Moreover, the instant invention provides a significant discovery made using a gemifloxacin compound against *Mycoplasma*, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein. Gemifloxacin compounds are valuable compounds for the treatment of bacterial infection caused by a range of *Mycoplasma* pathogens, including those resistant to usual oral therapy, thereby filling another unmet medical need.

The incidence of pneumococci resistant to penicillin G and other ß-lactam and non-ß-lactam compounds has increased worldwide at an alarming rate, including in the U.S. Major foci of infections currently include South Africa, Spain, Central and Eastern Europe, and parts of Asia (P.C. Appelbaum, *Clin. Infect. Dis.* 15:77-83, 1992; Friedland, et al., *Pediatr. Infect. Dis.* 11:433-435, 1992; Friedland, et al., *N. Engl. J. Med.* 331:377-382, 1994; Jacobs, et al., *Clin. Infect. Dis.* 15:119-127, 1992 and Jacobs, et al., *Rev. Med. Microbiol.* 6:77-93, 1995). In the U.S. a recent survey has shown an increase in resistance to penicillin from <5% before 1989 (including <0.02% of isolates with MICs ≥2.0 μg/ml) to 6.6% in 1991-1992 (with 1.3% of isolates with MICs ≥2.0 μg/ml) (Brieman, et al., *J. Am. Med. Assoc.* 271:1831-1835, 1994). In another more recent survey, 23.6% (360) of 1527 clinically significant pneumococcal isolates were not susceptible to penicillin (Doern, et al., *Antimicrob. Agents Chemother.* 40:1208-1213, 1996). It is also important to note the high rates of isolation of penicillin intermediate and resistant pneumococci (approximately 30%) in middle ear fluids from patients with refractory otitis media, compared to other isolation sites (Block, et al., *Pediatr. Infect. Dis.* 14:751-759, 1995). The problem of drug-resistant pneumococci is compounded by the ability of resistant clones to spread from country to country, and from continent to continent (McDougal, et al., *Antimicrob. Agents Chemother.* 36:2176-2184, 1992; Munoz, et al., *Clin. Infect. Dis.* 15:112-118, 1992).

There is an urgent need of oral compounds for out-patient treatment of otitis media and respiratory tract infections caused by penicillin intermediate and resistant pneumococci (Friedland, et al., *Pediatr. Infect. Dis.* 11:433-435, 1992; Friedland, et al., *N. Engl. J. Med.* 331:377-382, 1994; M.R. Jacobs, *Clin. Infect. Dis.* 15:119-127, 1992; and Jacobs, et al., *Rev. Med. Microbiol.* 6:77-93, 1995). Available quinolones such as ciprofloxacin and ofloxacin yield moderate in vitro activity against pneumococci, with MICs clustering around the breakpoints (Spangler, et al., *Antimicrob. Agents Chemother.* 36:856-859, 1992; and Spangler, et al., *J. Antimicrob. Chemother.* 31:273-280, 1993). Gemifloxacin (SB-265805) is a new broad-spectrum

fluoronaphthyridone carboxylic acid with a novel pyrrolidone substituent (Cormican, et al., *Antimicrob. Agents Chemother.* 41:204-211, 1997; Hohl, et al., *Clin. Microbiol. Infect.* 4:280-284, 1998; and Oh, et al., *Antimicrob. Agents Chemother.* 40:1564-1568, 1996). Previous preliminary studies (Cormican, et al., *Antimicrob. Agents Chemother.* 41:204-211, 1997; Hohl, et al., *Clin. Microbiol. Infect.* 4:280-284, 1998; and Oh, et al., *Antimicrob. Agents Chemother.* 40:1564-1568, 1996) have shown that this compound is very active against pneumococci. This study further examined the antipneumococcal activity of gemifloxacin compared to ciprofloxacin, levofloxacin, sparfloxacin, grepafloxacin, trovafloxacin, amoxicillin, cefuroxime, azithromycin and clarithromycin by i) agar dilution testing of 234 quinolone susceptible and resistant strains; ii) examination of resistance mechanisms in quinolone resistant strains; iii) time-kill testing of 12 strains; iv) examination of the post-antibiotic effect (PAE) of drugs against 6 strains.

Further provided herein is a significant discovery made using a gemifloxacin compound against a range of penicillin susceptible and resistant pneumococci by agar dilution, microdilution, time-kill and post-antibiotic effect methodology. Against 64 penicillin susceptible, 68 intermediate and 75 resistant pneumococci (all quinolone susceptible), agar dilution $MIC_{50}/_{90}$ values (μg/ml) were as follows: gemifloxacin, 0.03/0.06; ciprofloxacin, 1.0/4.0; levofloxacin, 1.0/2.0; sparfloxacin, 0.5/0.5; grepafloxacin, 0.125/0.5; trovafloxacin, 0.125/0.25; amoxicillin, 0.016/0.06 (penicillin susceptible), 0.125/1.0 (penicillin intermediate), 2.0/4.0 (penicillin resistant); cefuroxime, 0.03/0.25 (penicillin susceptible), 0.5/2.0 (penicillin intermediate), 8.0/16.0 (penicillin resistant); azithromycin, 0.125/0.5 (penicillin susceptible), 0.125/>128.0 (penicillin intermediate), 4.0/>128.0 (penicillin resistant); clarithromycin, 0.03/0.06 (penicillin susceptible), 0.03/32.0 (penicillin intermediate), 2.0/>128.0 (penicillin resistant). Against 28 strains with ciprofloxacin MICs ≥8 μg/ml, gemifloxacin had the lowest MICs (0.03-1.0 μg/ml, MIC_{90} 0.5 μg/ml), compared with MICs ranging between 0.25 to >32.0 μg/ml)(MIC_{90}s 4.0->32.0 μg/ml) for the other quinolones. Resistance in these 28 strains was associated with mutations in parC, gyrA, parE, and/or gyrB or efflux, with some strains having multiple resistance mechanisms. For 12 penicillin susceptible and resistant pneumococcal strains (2 quinolone resistant), time-kill results showed that levofloxacin at the MIC, gemifloxacin and sparfloxacin at 2 x MIC and ciprofloxacin, grepafloxacin and trovafloxacin at 4 x MIC, were bactericidal after 24 h. Gemifloxacin was uniformly bactericidal after 24 h at ≤0.5 μg/ml. Various degrees of 90% and 99% killing by all quinolones was detected after 3 h. Gemifloxacin and trovafloxacin were both bactericidal at the microbroth MIC for the two quinolone resistant pneumococcal strains. Amoxicillin, at 2 x MIC and cefuroxime at 4 x MIC, were bactericidal after 24 h, with some degree of killing at earlier time periods. By contrast, macrolides gave slower killing against the 7

susceptible strains tested, with 99.9% killing of all strains at 2-4 x MIC after 24 h. Post-antibiotic effects for 5 quinolone susceptible strains were similar (0.3-3.0 h) for all quinolones tested, and significant quinolone PAEs were found for the quinolone-resistant strain.

Recent surveillance in Canada indicated that the incidence of *S.pneumoniae* with reduced susceptibility to fluoroquinolone drugs is gradually increasing. Thus, a need existed for methods of treating these diseases. In an effort to make such methods, among other things, studies were designed to evaluate the activity of gemifloxacin against *S.pneumoniae* with reduced susceptibility to ciprofloxacin.

Also provided herein is a significant discovery made using a gemifloxacin compound against strains *Streptococcus pneumoniae*, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein. Gemifloxacin compounds are valuable compounds for the treatment of infection caused by a range of *Streptococcus pneumoniae* strains, including those resistant to usual oral therapy, thereby filling yet another unmet medical need.

Gemifloxacin (herein "GFX" or "gemifloxacin") is possesses potent gram positive activity, is active against penicillin susceptible and resistant *S. pneumoniae* and is also active against ciprofloxacin (herein "CFX" or "ciprofloxacin") and trovafloxacin (herein "TFX" or "trovafloxacin") resistant isolates.

Recent surveillance in Canada indicated that the incidence of *S.pneumoniae* with reduced susceptibility to fluoroquinolone drugs is gradually increasing. Thus, a need existed for methods of treating these diseases. In an effort to make such methods, among other things, studies were designed to evaluate the activity of gemifloxacin against *S.pneumoniae* with reduced susceptibility to ciprofloxacin.

Still further provided herein is a significant discovery made using a gemifloxacin compound against strains of *Streptococcus pneumoniae*, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein. Gemifloxacin compounds are valuable compounds for the treatment of infection caused by a range of *Streptococcus pneumoniae* strains, including those resistant to usual oral therapy, thereby filling an unmet medical need.

Another significant discovery, provided herein, made using a gemifloxacin compound against quinolone-resistant pneumocooci, demonstrated that the activity of a gemifloxacin compound used was superior to a number of quinolones as described in more detail herein. In light of this showing, it is clear that gemifloxacin compounds are valuable compounds for the

treatment of infections caused by a range of pneumococcal pathogens, including those resistant to usual oral therapy, thereby filling an unmet medical need.

The incidence of pneumococci resistant to penicillin G and other β-lactam and non-β-lactam compounds has increased worldwide at an alarming rate, including in the USA. Major foci of infections currently include South Africa, Spain, Central and Eastern Europe, and parts of Asia (P.C. Appelbaum, *Clin. Infect. Dis.* 15:77-83, 1992; Friedland, et al., *Pediatr. Infect. Dis. J.* 11:433-435, 1992; Friedland, et al., *N. Engl. J. Med.*, 331:377-382, 1994; M.R. Jacobs, *Clin. Infect. Dis.* 15:119-127, 1992; Jacobs, et al., *Rev. Med. Microbiol.* 6:77-93, 1995). In the USA, a recent survey has shown an increase in resistance to penicillin from <5% before 1989 (including <0.02% of isolates with MICs ≥2.0 μg/ml) to 6.6% in 1991–1992 (with 1.3% of isolates with MICs ≥2.0 μg/ml) (Breiman, et al., *JAMA* 271:1831-1835, 1994). In another more recent survey, 23.6% (360) of 1527 clinically significant pneumococcal isolates were not susceptible to penicillin (Doern, et al., *Antimicrob. Agents Chemother.* 40:1208-1213, 1996). It is also important to note the high rates of isolation of penicillin-intermediate and -resistant pneumococci (approximately 30%) in middle ear fluids from patients with refractory otitis media, compared to other isolation sites (Block, et al., *Pediatr. Infect. Dis.* 14:751-759, 1995). The problem of drug-resistant pneumococci is compounded by the ability of resistant clones to spread from country to country, and from continent to continent (McDougal, et al., *Antimicrob. Agents Chemother.* 36:2176-2184, 1992; Munoz, et al., *Clin. Infect. Dis.* 15:112-118, 1992).

There is an urgent need of oral compounds for out-patient treatment of otitis media and respiratory tract infections caused by penicillin-intermediate and -resistant pneumococci (Friedland, et al., *Pediatr. Infect. Dis.* 11:433-435, 1992; Friedland, et al., *N. Engl. J. Med.* 331:377-382, 1994); M.R. Jacobs, *Clin. Infect. Dis.* 15:119-127, 1992; and Jacobs, et al., *Rev. Med. Microbiol.* 6:77-93, 1995). Available quinolones such as ciprofloxacin and ofloxacin yield moderate *in vitro* activity against pneumococci, with MICs clustering around the breakpoints (Pankuch, et al., *J. Antimicrob. Chemother.* 35:230-232, 1995; Spangler, et al., *Antimicrob. Agents Chemother.* 36:856-859, 1992; Spangler, et al., *J. Antimicrob. Chemother.* 31:273-280, 1993). Gemifloxacin (SB-265805) is a new broad-spectrum fluoronaphthyridone carboxylic acid with a novel pyrrolidone substituent (Cormican, et al., *Antimicrobiol. Agents Chemother.* 41:204-211, 1997; Hohl, et al., *Clin. Microbiol. Infect.* 4:280-284, 1998; Oh, et al., *Antimicrob. Agents Chemother.* 40:1564-1568, 1996). Previous preliminary studies (Cormican, et al., *Antimicrobiol. Agents Chemother.* 41:204-211, 1997; Hohl, et al., *Clin. Microbiol. Infect.* 4:280-284, 1998; Oh, et al., *Antimicrob. Agents Chemother.* 40:1564-1568, 1996) have shown that this compound is very active against pneumococci. This study further examined the antipneumococcal activity of

gemifloxacin compared to ciprofloxacin, levofloxacin, sparfloxacin, grepafloxacin, trovafloxacin, amoxicillin, cefuroxime, azithromycin and clarithromycin. It also examined resistance mechanisms in quinolone-resistant strains.

Although development of an effective vaccine against *Haemophilus influenzae* type b has led to disappearance of this organism in many parts of the world, its place has been taken by untypeable *H. influenzae* strains. The latter organisms (followed by *Streptococcus pneumoniae* and *Moraxella catarrhalis*) are now considered to be the leading cause of acute exacerbations of chronic bronchitis, and an important cause, together with *S. pneumoniae* and *M. catarrhalis*, of acute otitis media, sinusitis and community-acquired respiratory tract infections (Fang, et al., *Medicine (Baltimore)* 69:307-316, 1990; Hoberman, et al., *Pediatr. Infect. Dis.* 10:955-962, 1996; Jacobs, et al., *Antimicrob. Agents Chemother,* In press; and Zeckel, et al., *Clin. Ther.* 14:214-229, 1992).

Current recommendations by the NCCLS for use of *Haemophilus* Test Medium (HTM) for *Haemophilus* susceptibility testing have been complicated by difficulty in commercial manufacture of this medium, and its short half-life when made in-house. Reliable *Haemophilus* susceptibility testing with HTM requires use of freshly made medium used within 3 weeks of making (*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically,* 3rd Edition, NCCLS, Wayne, PA, 1997).

Previous preliminary studies have shown that this compound is very active against *Haemophilus* and *Moraxella* (Cormican, et al., *Antimicrob. Agents Chemother.* 41:204-211, 1997; Hohl, et al., *Clin. Microbiol. Infect.* 4:280-284, 1998; and Oh, et al., *Antimicrobial Agents Chemother.* 40:1564-1568, 1996).

In light of these studies, provided herein is a significant discovery made using a gemifloxacin compound against nine rare clinical strains of *Haemophilus influenzae* from Europe with increased ciprofloxacin MICs were tested for *in vitro* activity (MICs) of gemifloxacin (SB-265805), ciprofloxacin, levofloxacin, sparfloxacin, grepafloxacin and trovafloxacin and checked for mutations in *gyrA, parC, gyrB* and *parE,* demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein. Gemifloxacin compounds are valuable compounds for the treatment of infections caused by a range of *Haemophilus influenzae* strains, including those resistant to usual oral therapy, thereby filling an unmet medical need.

Also provided herein is a significant discovery made using a gemifloxacin compound against ciprofloxacin-sensitive and ciprofloxacin-resistant pneumococci, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as

described in more detail herein. Gemifloxacin compounds are valuable compounds for the treatment of infections caused by a range of bacterial pathogens, including those resistant to usual oral therapy, thereby filling an unmet medical need.

The incidence of pneumococci resistant to penicillin G and other β-lactam and non-β-lactam compounds has increased worldwide at an alarming rate, including in the USA Major foci of resistance currently include South Africa, Spain, Central and Eastern Europe, and parts of Asia (P.C. Appelbaum, *Clin. Infect. Dis.* 15:77-83, 1992). In the USA there has been an increase in resistance to penicillin from <5% before 1989 (including <0.02% of isolates with MICs ≥2.0 μg/ml) to 6.6% in 1991–1992 (with 1.3% of isolates with MICs ≥2.0 μg/ml), (Breiman, et al., *JAMA* 271:1831-1835, 1994) and to 23.6% (360) of 1527 strains during 1994–1995 (Doern, et al., *Antimicrob. Agents Chemother.* 40:1208-1213, 1996). It is also important to note the high rates of isolation of penicillin-intermediate and -resistant pneumococci (approximately 30%) in middle ear fluids from patients with refractory otitis media, compared to other isolation sites (Block, et al., *Pediatr. Infect. Dis.* 14:751-759, 1995).

There is an urgent need of oral compounds for out-patient treatment of otitis media and other respiratory tract infections caused by penicillin-intermediate and -resistant pneumococci (Friedland, et al., *N. Eng. J. Med.,* 331:377-382, 1994 and M.R. Jacobs, *Clin. Infect. Dis.* 15:119-127, 1992). Older quinolones such as ciprofloxacin and ofloxacin yield moderate *in vitro* activity against pneumococci, with MICs clustering around the breakpoints (Pankuch, et al., *J. Antimicrob. Chemother.* 35:230-232, 1995). Methods for routine susceptibility testing of pneumococci include broth microdilution and disk diffusion (the methods recommended by the NCCLS, agar dilution and E-test (*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 4th Edition, NCCLS, Villanova, PA, 1997 and *Performance Standards for Antimicrobial Disk Susceptibility Tests,* 6th Edition, NCCLS, Villanova, PA, 1997). NCCLS recommends incubation of microdilution MICs in ambient air, but disk diffusion in CO_2, (*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 4th Edition, NCCLS, Villanova, PA, 1997 and *Performance Standards for Antimicrobial Disk Susceptibility Tests,* 6th Edition, NCCLS, Villanova, PA, 1997) while the manufacturer of the E-test recommends incubation in CO_2. There is no standard recommendation for agar dilution pneumococcal MIC testing methodology, although the method has been extensively used in the art (M.R. Jacobs, *Clin. Infect. Dis.* 15:119-127, 1992; Pankuch et al., *J. Antimicrob. Chemother.* 35:230-232, 1995; and Clark, et al., *J. Clin. Microbiol.* 36:3579-3584, 1998).

If certain new compounds are to be tested for anti-pneumococcal activity in the clinical laboratory, methodology should be standardized. The current study used microdilution and agar dilution (air), E-test (air and CO_2) and disk diffusion (air and CO_2) to test activity of gemifloxacin (SB-265805), a new fluoronaphthyridone with a novel pyrrolidone substituent, with good Gram positive and negative activity, (Oh, et al., *Antimicrob. Agents Chemother.* 40:1564-1568, 1996; Cormican, et al., *Antimicrob. Agents Chemother.* 41:204-211, 1997; Hohl, et al., *Clin. Microbiol. Infect.* 4:280-284, 1998; Kelly, et al., *Program and Abstracts of the Thirty-Eighth Interscience Conference on Antimicrobial Agents and Chemotherapy,* San Diego, CA, USA 1998. American Society for Microbiology: Washington, DC 1998, pages 254, Abstract F-87) against 200 pneumococci, including those with raised penicillin and quinolone MICs.

Moreover, provided herein is a significant discovery made using a gemifloxacin compound against *Streptococcus pneumoniae* strains with elevated MICs to ciprofloxacin, demonstrating the activity of the gemifloxacin compound used was superior to a number of quinolones as described in more detail herein. Gemifloxacin compounds are valuable compounds for the treatment of infections by bacterial strains with elevated MICs to ciprofloxacin, particularly *Streptococcus pneumoniae* strains, thereby filling an unmet medical need.

SUMMARY OF THE INVENTION

An object of the invention is a method for modulating metabolism of maxillary sinus pathogenic bacteria comprising the step of contacting maxillary sinus pathogenic bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said maxillary sinus pathogenic bacteria is selected from the group consisting of: a bacterial strains isolated from acute or chronic maxillary sinusitis; and a maxillary sinus isolate of *S. aureus*, *S. pneumoniae, Haemophilus* spp., *M. catarrhalis*, and anaerobic strain or non-fermentative Gram negative bacilli, *Neisseria meningitidis* and β-haemolytic *Streptococcus*.

Also provided by the invention is a method of treating or preventing a bacterial infection by maxillary sinus pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with maxillary sinus pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: a bacterial strain isolated from acute or chronic maxillary sinusitis; a maxillary sinus isolate of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus* spp., *Moraxella catarrhalis*, an anaerobic strain or non-fermentative Gram negative bacilli, *Neisseria meningitidis*, β-haemolytic *Streptococcus*, *Haemophilus influenzae*, an *Enterobacteriaceae*, a non-fermentative Gram negative bacilli, *Streptococcus pneumoniae*, *Streptococcus pyogenes*, a methicillin-resistant *Staphylococcus* spp., *Legionella pneumophila*, *Mycoplasma* spp. and *Chlamydia* spp., *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Peptostreptococcus*, *Bacteroides* spp., and *Bacteroides urealyticus*.

An object of the invention is a method for modulating metabolism of anaerobic pathogenic bacteria comprising the step of contacting anaerobic pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said anaerobic pathogenic bacteria is selected from the group consisting of: a member of the genus *Peptostreptococci*, a *Peptostreptococci asaccharolyticus*, a *Peptostreptococci magnus*, a *Peptostreptococci micros*, a *Peptostreptococci prevotii*, a *Porphyromonas asaccharolytica*, a *Porphyromonas canoris*, a *Porphyromonas gingivalis*, a *Porphyromonas macaccae*, a member of the genus *Actinomyces*, an *Actinomyces israelii*, an *Actinomyces odontolyticus*, a member of the genus *Clostridium*, a *Clostridium innocuum*, a *Clostridium clostridioforme*, a *Clostridium difficile*, a member of the genus *Anaerobiospirillum*, a *Bacteroides tectum*, a *Bacteroides ureolyticus*, a *Bacteroides gracilis* (*Campylobacter gracilis*), a *Prevotella intermedia*, a *Prevotella heparinolytica*, a *Prevotella oris-buccae*, a *Prevotella bivia*, a *Prevotella melaninogenica*, a member of the genus *Fusobacterium*, a *Fusobacterium naviforme*, a *Fusobacterium necrophorum*, a *Fusobacterium varium*, a *Fusobacterium ulcerans*, a *Fusobacterium russii*, a member of the genus *Bilophila*, a *Bilophila wadsworthia*.

Also provided by the invention is a method of treating or preventing a bacterial infection by anaerobic pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with anaerobic pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: a member of the genus *Peptostreptococci*, a

Peptostreptococci asaccharolyticus, a *Peptostreptococci magnus*, a *Peptostreptococci micros*, a *Peptostreptococci prevotii*, a member of the genus *Porphyromonas*, a *Porphyromonas asaccharolytica*, a *Porphyromonas canoris*, a *Porphyromonas gingivalis*, a *Porphyromonas macaccae*, a member of the genus *Actinomyces*, an *Actinomyces israelii*, an *Actinomyces odontolyticus*, a member of the genus *Clostridium*, a *Clostridium innocuum*, a *Clostridium clostridioforme*, a *Clostridium difficile*, a member of the genus *Anaerobiospirillum*, a member of the genus *Bacteroides*, a *Bacteroides tectum*, a *Bacteroides ureolyticus*, a *Bacteroides gracilis* (*Campylobacter gracilis*), a member of the genus *Prevotella*, a *Prevotella intermedia*, a *Prevotella heparinolytica*, a *Prevotella oris-buccae*, a *Prevotella bivia*, a *Prevotella melaninogenica*, a member of the genus *Fusobacterium*, a *Fusobacterium naviforme*, a *Fusobacterium necrophorum*, a *Fusobacterium varium*, a *Fusobacterium ulcerans*, a *Fusobacterium russii*, a member of the genus *Bilophila*, a *Bilophila wadsworthia*.

An object of the invention is a method for modulating metabolism of atypical upper respiratory pathogenic bacteria comprising the step of contacting atypical upper respiratory pathogenic bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said atypical upper respiratory pathogenic bacteria is selected from the group consisting of: a member of the genus *Legionella*, a member of the genus, *Pseudomonas*, *Pseudomonas aeruginosa* strain, a *Legionella pneumophila* strain, a *Legionella pneumophila* serogroup 1, a *Legionella pneumophila* serogroup 2, a *Legionella pneumophila* serogroup 3, a *Legionella pneumophila* serogroup 4, a *Legionella pneumophila* serogroup 5, a *Legionella pneumophila* serogroup 6, a *Legionella pneumophila* serogroup 7, a *Legionella pneumophila* serogroup 8, a *Legionella dumoffii* strain, a *Legionella longbeacheae* strain, a *Legionella micdadei* strain, a *Legionella oakridgensis* strain, a *Legionella feelei* strain, a *Legionella anisa* strain, a *Legionella sainthelensi* strain, a *Legionella bozemanii* strain, a *Legionella gormanii* strain, a *Legionella wadsworthii* strain, a *Legionella jordanis* strain and a *Legionella gormanii* strain.

Also provided by the invention is a method of treating or preventing a bacterial infection by atypical upper respiratory pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with atypical upper respiratory pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: a member of the genus *Legionella*, a member of the

genus, *Pseudomonas, Pseudomonas aeruginosa* strain, a *L. pneumophila* strain, a *L. pneumophila* serogroup 1, a *L. pneumophila* serogroup 2, a *L. pneumophila* serogroup 3, a *L. pneumophila* serogroup 4, a *L. pneumophila* serogroup 5, a *L. pneumophila* serogroup 6, a *L. pneumophila* serogroup 7, a *L. pneumophila* serogroup 8, a *L. dumoffii* strain, a *L. longbeacheae* strain, a L. micdadei strain, a L. oakridgensis strain, a *L. feelei* strain, a *L. anisa* strain, a *L. sainthelensi* strain, a *L. bozemanii* strain, a *L. gormanii* strain, a *L. wadsworthii* strain, a *L. jordanis* strain and a *L. gormanii* strain.

A further embodiment of the invention is method for modulating metabolism of atypical upper respiratory pathogenic bacteria comprising the step of contacting atypical upper respiratory pathogenic bacteria with an antibacterially effective amount of a composition comprising a compound selected from the group consisting of: gemifloxacin, ofloxacin, levofloxacin, trovafloxacin, azithromycin, moxifloxacin, ciprofloxacin, clarithromycin, rifampicin and erythromycin; or an antibacterially effective derivative of any of these compounds.

A still further embodiment of the invention is a method of treating or preventing a bacterial infection by atypical upper respiratory pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a compound selected from the group consisting of: gemifloxacin, ofloxacin, levofloxacin, trovafloxacin, azithromycin, moxifloxacin, ciprofloxacin, clarithromycin, rifampicin and erythromycin; or an antibacterially effective derivative of any of these compounds, to a mammal suspected of having or being at risk of having an infection with atypical upper respiratory pathogenic bacteria.

It is preferred in the methods of the invention that said contacting is performed once daily.

An object of the invention is a method for modulating metabolism of pathogenic Mycoplasma bacteria comprising the step of contacting pathogenic Mycoplasma bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said pathogenic Mycoplasma bacteria is selected from the group consisting of: *Mycoplasma pneumoniae, M. hominis, M. fermentans, M. genitalium, M. penetrans* and *Ureaplasma urealyticum*.

Also provided by the invention is a method of treating or preventing a bacterial infection by pathogenic Mycoplasma bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with pathogenic Mycoplasma bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: *Mycoplasma pneumoniae*, *M. hominis*, *M. fermentans*, *M. genitalium*, *M. penetrans* and *Ureaplasma urealyticum*.

An object of the invention is a method for modulating metabolism of pneumococcal pathogenic bacteria comprising the step of contacting pneumococcal pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said pneumococcal pathogenic bacteria is selected from the group consisting of: bacteria comprising a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB; bacteria comprising a mutation in ParC at S79-F or Y, D83-N, R95-C, or K137-N; bacteria comprising a mutation in gyrA at S83-A, C, F, or Y; E87-K; or S116-G; bacteria comprising a mutation in parE at D435-N or I460-V; bacteria comprising a mutation in gyrB at D435-N or E474-K; bacteria comprising at least four mutations in a QRDR or parC, gyrA, parE, and gyrB; bacteria comprising a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in ParC at S79-F or Y, D83-N, R95-C, or K137-N; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in gyrA at S83-A, C, F, or Y; E87-K; or S116-G; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in parE at D435-N or I460-V; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in gyrB at D435-N or E474-K; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise at least four mutations in a QRDR or parC, gyrA, parE, and gyrB; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB; *Streptococcus pneumoniae* bacteria comprising a mutation in ParC at S79-F or Y, D83-N, R95-C, or K137-N; *Streptococcus pneumoniae* bacteria comprising a mutation in gyrA at S83-A, C, F, or Y; E87-K; or S116-G; *Streptococcus pneumoniae* bacteria comprising a mutation in parE at D435-N or I460-V; *Streptococcus pneumoniae* bacteria

comprising a mutation in gyrB at D435-N or E474-K; *Streptococcus pneumoniae* bacteria comprising at least four mutations in a QRDR or parC, gyrA, parE, and gyrB; and *Streptococcus pneumoniae* bacteria comprising a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB.

Also provided by the invention is a method of treating or preventing a bacterial infection by pneumococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with pneumococcal pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: bacteria comprising a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB; bacteria comprising a mutation in ParC at S79-F or Y, D83-N, R95-C, or K137-N; bacteria comprising a mutation in gyrA at S83-A, C, F, or Y; E87-K; or S116-G; bacteria comprising a mutation in parE at D435-N or I460-V; bacteria comprising a mutation in gyrB at D435-N or E474-K; bacteria comprising at least four mutations in a QRDR or parC, gyrA, parE, and gyrB; bacteria comprising a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in ParC at S79-F or Y, D83-N, R95-C, or K137-N; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in gyrA at S83-A, C, F, or Y; E87-K; or S116-G; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in parE at D435-N or I460-V; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in gyrB at D435-N or E474-K; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise at least four mutations in a QRDR or parC, gyrA, parE, and gyrB; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB; *Streptococcus pneumoniae* bacteria comprising a mutation in ParC at S79-F or Y, D83-N, R95-C, or K137-N; *Streptococcus pneumoniae* bacteria comprising a mutation in gyrA at S83-A, C, F, or

Y; E87-K; or S116-G; *Streptococcus pneumoniae* bacteria comprising a mutation in parE at D435-N or I460-V; *Streptococcus pneumoniae* bacteria comprising a mutation in gyrB at D435-N or E474-K; *Streptococcus pneumoniae* bacteria comprising at least four mutations in a QRDR or parC, gyrA, parE, and gyrB; and *Streptococcus pneumoniae* bacteria comprising a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB.

Also provided is a method for modulating the activity of a topoisomerase comprising a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA or parE or gyrB.

It is preferred in the methods of the invention that said mutation in ParC is at S79-F or Y, D83-N, R95-C, or K137-N; said mutation in gyrA is at S83-A, C, F, or Y; E87-K; or S116-G; said mutation in parE is at D435-N or I460-V; or said mutation in gyrB is at D435-N or E474-K.

An object of the invention is a method for modulating metabolism of fluoroquinolone resistant pathogenic bacteria comprising the step of contacting fluoroquinolone resistant pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said pathogenic bacteria is selected from the group consisting of: a ciprofloxacin resistant strain of *S. pneumoniae, S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae,* a trovafloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a fluoroquinolone resistant strain of *S. pneumoniae,* a fluoroquinolone resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, and a fluoroquinolone resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region.

Also provided by the invention is a method of treating or preventing a bacterial infection by fluoroquinolone resistant pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with fluoroquinolone resistant pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: a ciprofloxacin resistant strain of *S. pneumoniae, S.*

pneumoniae having a topisomerase *IV* (*parC*) mutation in the QRDR region, *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae,* a trovafloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a fluoroquinolone resistant strain of *S. pneumoniae,* a fluoroquinolone resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, and a fluoroquinolone resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region.

An object of the invention is a method for modulating metabolism of fluoroquinolone resistant pathogenic bacteria comprising the step of contacting fluoroquinolone resistant pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said pathogenic bacteria is selected from the group consisting of: a ciprofloxacin resistant strain of *S. pneumoniae, S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae,* a trovafloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a fluoroquinolone resistant strain of *S. pneumoniae,* a fluoroquinolone resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, and a fluoroquinolone resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region.

Also provided by the invention is a method of treating or preventing a bacterial infection by fluoroquinolone resistant pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with fluoroquinolone resistant pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: a ciprofloxacin resistant strain of *S. pneumoniae*, *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae*, a trovafloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a fluoroquinolone resistant strain of *S. pneumoniae*, a fluoroquinolone resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, and a fluoroquinolone resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region.

The invention also provides a method for modulating metabolism of fluoroquinolone resistant pathogenic bacteria comprising the step of contacting fluoroquinolone resistant pathogenic bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, and a ciprofloxacin compound, or antibacterially effective derivatives thereof either compound or both compounds.

Further provided by the invention is a method of treating or preventing a bacterial infection by fluoroquinolone resistant pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound and a ciprofloxacin compound to a mammal suspected of having or being at risk of having an infection with fluoroquinolone resistant pathogenic bacteria.

A still further method of the invention is a method for modulating metabolism of fluoroquinolone resistant pathogenic bacteria comprising the step of contacting fluoroquinolone resistant pathogenic bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, followed by a ciprofloxicin compound, or antibacterially effective derivatives thereof either compound or both compounds.

Another embodiment of the invention is a method of treating or preventing a bacterial infection by fluoroquinolone resistant pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound followed by a ciprofloxicin compound to a mammal suspected of having or being at risk of having an infection with fluoroquinolone resistant pathogenic bacteria.

An object of the invention is a method for modulating metabolism of quinolone-resistant pneumococcal pathogenic bacteria comprising the step of contacting quinolone-resistant pneumococcal pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said quinolone-resistant pneumococcal pathogenic bacteria is selected from the group consisting of: a pneumococcal strain comprising a mutation in the quinolone resistance-determining region (QRDR) of *parC* and/or *gyrA*; a pneumococcal strain comprising a mutation in ParC said mutation comprising S79→F and/or Y, D83→G and/or N, N91→D, R95→C, and/or K137→N; a pneumococcal strain comprising a mutation in GyrA said mutation comprising S81→A, C, F, or Y; E85→K; and/or S114→G; a pneumococcal strain comprising a mutation in ParE said mutation comprising D435→N and/or I460→V; a pneumococcal strain comprising a mutation in GyrB said mutation comprising D435→N and/or E474→K; a pneumococcal strain comprising a mutation in comprising three or four mutations in a QRDRs of *parC*, *gyrA*, *parE*, and/or *gyrB*; a pneumococcal strain comprising a mutation in comprising three or four mutations in a QRDRs of *parC*, *gyrA*, *parE*, and/or *gyrB*, any of which are resistant to ciprofloxacin, levofloxacin, or sparfloxacin; and a pneumococcal strain comprising a mutation in comprising three or four mutations in a QRDRs of *parC*, *gyrA*, *parE*, and/or *gyrB*, any of which also comprising an efflux mechanism of quinolone resistance.

Also provided by the invention is a method of treating or preventing a bacterial infection by quinolone-resistant pneumococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with quinolone-resistant pneumococcal pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: a pneumococcal strain comprising a mutation in the quinolone resistance-determining region (QRDR) of *parC* and/or *gyrA*; a pneumococcal strain comprising a mutation in ParC said mutation comprising S79→F and/or Y, D83→G and/or N, N91→D, R95→C, and/or K137→N; a pneumococcal strain comprising a mutation in GyrA said mutation comprising S81→A, C, F, and/or Y; E85→K; and/or S114→G; a pneumococcal strain comprising a mutation in ParE said mutation comprising D435→N and/or I460→V; a pneumococcal strain comprising a mutation in GyrB said mutation comprising D435→N

and/or E474→K; a pneumococcal strain comprising a mutation in comprising three or four mutations in a QRDRs of *parC*, *gyrA*, *parE*, and/or *gyrB*; a pneumococcal strain comprising a mutation in comprising three or four mutations in a QRDRs of *parC*, *gyrA*, *parE*, and/or *gyrB*, any of which are resistant to ciprofloxacin, levofloxacin, or sparfloxacin; and a pneumococcal strain comprising a mutation in comprising three or four mutations in a QRDRs of *parC*, *gyrA*, *parE*, and/or *gyrB*, any of which also comprising an efflux mechanism of quinolone resistance.

An object of the invention is a method for modulating metabolism of a rare *Haemophilus influenzae* strain comprising the step of contacting a rare *Haemophilus influenzae* strain with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said rare pathogenic H. influenzae strain is selected from the group consisting of: bacteria comprising a mutation set forth in Table 25 or 26; a *Haemophilus influenzae* strain set forth in Table 25 or 26; bacteria of the genus *Haemophilus* comprising a mutation set forth in Table 25 or 26; and bacteria of the species *Haemophilus influenzae* comprising a mutation set forth in Table 25 or 26.

Also provided by the invention is a method of treating or preventing a bacterial infection by a rare pathogenic H. influenzae strain comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with a rare pathogenic H. influenzae strain.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: bacteria comprising a mutation set forth in Table 25 or 26; a *Haemophilus influenzae* strain set forth in Table 25 or 26; bacteria of the genus *Haemophilus* comprising a mutation set forth in Table 25 or 26; and bacteria of the species *Haemophilus influenzae* comprising a mutation set forth in Table 25 or 26.

An object of the invention is a method for modulating metabolism of ciprofloxacin-resistant and/or ciprofloxicin-sensitive bacteria comprising the step of contacting ciprofloxacin-resistant and/or ciprofloxicin-sensitive bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said ciprofloxacin-resistant and/or ciprofloxicin-sensitive bacteria is selected from the group consisting of: ciprofloxacin-susceptible pneumococci having an MIC ≤4 μg/ml of ciprofloxacin; ciprofloxacin-resistant

pneumococci having an MIC ≥8 μg/ml of ciprofloxacin; ciprofloxacin-susceptible *Streptococcus pneumoniae* having an MIC ≤4 μg/ml of ciprofloxacin; and ciprofloxacin-resistant *Streptococcus pneumoniae* having an MIC ≥8 μg/ml of ciprofloxacin.

Also provided by the invention is a method of treating or preventing a bacterial infection by ciprofloxacin-resistant and/or ciprofloxicin-sensitive bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with ciprofloxacin-resistant and/or ciprofloxicin-sensitive bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: ciprofloxacin-susceptible pneumococci having an MIC 4 μg/ml of ciprofloxacin; ciprofloxacin-resistant pneumococci having an MIC ≥8 μg/ml of ciprofloxacin; ciprofloxacin-susceptible *Streptococcus pneumoniae* having an MIC ≤4 μg/ml of ciprofloxacin; and ciprofloxacin-resistant *Streptococcus pneumoniae* having an MIC ≥8 μg/ml of ciprofloxacin.

Another object of the invention is a method for modulating metabolism of ciprofloxacin-resistant or trovafloxacin-resistant pathogenic bacteria comprising the step of contacting ciprofloxacin-resistant or trovafloxacin-resistant pathogenic bacteria with an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound, or an antibacterially effective derivative thereof.

A further object of the invention is a method wherein said ciprofloxacin-resistant or trovafloxacin-resistant pathogenic bacteria is selected from the group consisting of: a bacteria with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a respiratory tract pathogenic bacteria with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a member of the genus *Streptococcus* with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a *Streptococcus pneumoniae* strain with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a penicillin-resistant member of the genus *Streptococcus* with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, and a penicillin-resistant *Streptococcus pneumoniae* strain with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin.

Also provided by the invention is a method of treating or preventing a bacterial infection by ciprofloxacin-resistant or trovafloxacin-resistant pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal suspected of having or being at

risk of having an infection with ciprofloxacin-resistant or trovafloxacin-resistant pathogenic bacteria.

Further preferred methods are provided by the invention wherein said bacteria is selected from the group consisting of: a bacteria with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a respiratory tract pathogenic bacteria with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a member of the genus *Streptococcus* with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a *Streptococcus pneumoniae* strain with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a penicillin-resistant member of the genus *Streptococcus* with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, and a penicillin-resistant *Streptococcus pneumoniae* strain with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin.

A preferred method of the invention uses a gemifloxacin compound that is a gemifloxacin mesylate, particularly hydrates, especially hemihydrates and sesquihydrates thererof.

Another preferred method is provided wherein said modulating metabolism is inhibiting growth of said bacteria or killing said bacteria.

A further preferred method is provided wherein said contacting said bacteria comprises the further step of introducing said composition into a mammal, particularly a human.

A method is also provided using a gemifloxacin compound in veterinary applications, particularly in livestock.

Also provided are methods of making medicaments useful for the methods of the invention, particulary the methods of treatment of bacterial infections and methods of inhibiting bacteria.

Various changes and modifications within the spirit and scope of the disclosed invention will become readily apparent to those skilled in the art from reading the following descriptions and from reading the other parts of the present disclosure.

BRIEF DESRCIPTION OF THE DRAWINGS

Figure 1 shows a graphical depiction of agar dilution MICs obtained with ciprofloxacin compared with those obtained with gemifloxacin against 161 ciprofloxacin-susceptible 39 and ciprofloxacin-resistant strains

Figure 2 shows a graphical depiction of gemifloxacin zone diameters (mm) in CO_2 compared with microdilution MICs in air for all 200 strains

DESCRIPTION OF THE INVENTION

(A) Methods of Using Fluoroquinolones Against Maxillary Sinus Bacteria

The present invention provides, among other things, methods for using a composition comprising a gemifloxacin compound against maxillary sinus pathogenic bacteria, especially maxillary sinus strains of *S. aureus, S. pneumoniae, Haemophilus* spp., *M. catarrhalis*, certain anaerobic strains, non-fermentative Gram negative bacilli, *Neisseria meningitidis* and β-haemolytic *Streptococcus.*

As used herein "gemifloxacin compound(s)" means a compound having antibacterial activity described in patent application PCT/KR98/00051 published as WO 98/42705, or patent application EP 688772.

An aspect of this invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various maxillary sinus pathogens. An objective of these analyses was to determine minimum inhibitory concentrations (herein "MIC") of gemifloxacin, ciprofloxacin, ofloxacin, levofloxacin, trovafloxacin, grepafloxacin, moxifloxacin, sparfloxacin, amoxycillin and amoxycillin/clavulanic acid against a variety of strains such as *Haemophilus* spp. *S. pneumoniae* and *Moraxella catarrhalis*, isolated recently from acute or chronic maxillary sinus infections.

Gemifloxacin was compared to ciprofloxacin, ofloxacin, levofloxacin, trovafloxacin, grepafloxacin, moxifloxacin, sparfloxacin, amoxycillin and amoxycillin/clavulanic acid against a total of more than 250 recent isolates from acute or chronic maxillary sinusitis. MICs were determined by agar dilution techniques using standard NCCLS methodology. The activity of gemifloxacin (MIC_{90} 0.06 mg/L) was superior to ciprofloxacin, ofloxacin, levofloxacin, grepafloxacin, moxifloxacin and sparfloxacin (MIC_{90} ≥0.25 mg/L) against the *Streptococcus pneumoniae* isolates tested. Against *Moraxella catarrhalis* and *Haemophilus influenzae,* gemifloxacin and grepafloxacin (MIC_{90} ≤0.02 mg\L) were the most active antimicrobial agents tested. Against *Staphylococcus aureus*, gemifloxacin, trovafloxacin and moxifloxacin were more active (MIC_{90} 0.06 mg\L) than ciprofloxacin amoxycillin and amoxycillin/clavulanic acid (MIC_{90} ≥1 mg\L). A similar activity (MIC_{90} 0.25 mg\L) was observed with gemifloxacin and moxifloxacin against anaerobic strains tested. The activity of gemifloxacin was similar to ofloxacin, trovafloxacin, moxifloxacin and sparfloxacin (MIC_{90} 0.5 mg/L) against various other strains such as some *Enterobacteriaceae* or non-fermentative Gram negative bacilli. Combined with favourable pharmacokinetics in humans, gemifloxacin should be a valuable oral compound for the treatment of acute or chronic sinusitis caused by a range of respiratory

pathogens, including those resistant to usual oral therapy. The susceptibility results are presented in Tables 2–5.

These analyses showed that gemifloxacin is appreciably more potent than most fluoroquinolones against many Gram positive organisms, including *Streptococcus pneumoniae*, *Streptococcus pyogenes* and methicillin-resistant *Staphylococcus* spp. Gemifloxacin retains activity against a range of Gram negative bacilli, including those resistant to other antimicrobial agents. It also has potent activity against various anaerobic and atypical respiratory pathogens, such as *Legionella pneumophila*, *Mycoplasma* spp. and *Chlamydia* spp.

Against *S. pneumoniae*, gemifloxacin activity (MIC_{90} 0.06 mg/L) was similar to trovafloxacin, but superior to ciprofloxacin, ofloxacin, levofloxacin and sparfloxacin (MIC_{90} <0.5 mg/L) (Table 2). Against *S. aureus* sinus pathogens, gemifloxacin, moxifloxacin, trovafloxacin (MIC_{90} 0.06 mg/L) and sparfloxacin (MIC_{90} 0.12 mg/L) were the most active compounds tested. Ciprofloxacin, amoxycillin (MIC_{90} 1 mg/L) and amoxycillin/clavulanic acid (MIC_{90} 2 mg/L) were less active against *S. aureus* (Table 2).

H. influenzae strains were susceptible to gemifloxacin at a MIC_{90} of ≤0.02 mg/L (Table 3). This activity was significantly superior to ofloxacin, moxifloxacin, sparfloxacin, amoxycillin and amoxycillin/clavulanic acid. Against *Haemophilus parainfluenzae*, gemifloxacin (MIC_{90} 0.12 mg/L) was superior to ofloxacin (MIC_{90} 0.5 mg/L), moxifloxacin (MIC_{90} 0.5 mg/L), sparfloxacin (MIC_{90} 1 mg/L), amoxycillin (MIC_{90} 1 mg/L) and amoxycillin/clavulanic acid (MIC_{90} 0.5 mg/L).

Against *M. catarrhalis*, gemifloxacin and grepafloxacin (MIC_{90} ≤0.02 mg/L) were the most active compounds tested (Table 4). Gemifloxacin was significantly more potent than sparfloxacin, amoxycillin/clavulanic acid (MIC_{90} 0.5 mg/L) and amoxycillin (MIC_{90} 8 mg/L).

Against anaerobic strains, gemifloxacin (MIC_{90} 0.25 mg/L) and moxifloxacin (MIC_{90} 0.25 mg/L) were the most active agents tested (Table 5). The activity of gemifloxacin was significantly superior to ofloxacin (MIC_{90} 2 mg/L), trovafloxacin (MIC_{90} 4 mg/L), grepafloxacin (MIC_{90} 8 mg/L) and sparfloxacin (MIC_{90} 16 mg/L). Against various other streptococcal strains, gemifloxacin was as active as ofloxacin, trovafloxacin, moxifloxacin and sparfloxacin (MIC_{90} 0.5 mg/L).

Gemifloxacin shows a broad spectrum of antibacterial activity against a broad range of bacterial strains isolated from acute or chronic maxillary sinusitis.

The activity of gemifloxacin was higher than other agents tested against a broad range of maxillary sinus isolates, such as, for example, *S. aureus*, *Haemophilus* spp., *M. catarrhalis* and anaerobic strains. The overall *in vitro* activity of this compound is significantly greater

than ciprofloxacin, ofloxacin, levofloxacin and sparfloxacin against strains of *S. pneumoniae*. Gemifloxacin also has significant activity against *Haemophilus* spp., *M. catarrhalis*, some anaerobic strains and other various strains tested such as: non-fermentative Gram negative bacilli, *Neisseria meningitidis* and β-haemolytic *Streptococcus*. Combined with favourable pharmacokinetics in humans, gemifloxacin is a valuable oral compound for the treatment of acute or chronic sinusitis caused by microbial agents resistant to usual oral therapy.

Table 1. Test Strains Isolated from Maxillary Sinus Pathogens

Microrganism	No. of tested strains
Streptococcus pneumoniae	85
Haemophilus influenzae	45
Haemophilus parainfluenzae	10
Moraxella catarrhalis	45
Staphylococcus aureus	31
Anaerobes*	22
Other spp.†	15

*Including *Peptostreptococcus* and *Bacteroides* spp.

†Including beta-haemolytic *Streptococcus* and Gram negative rods

Table 2. Susceptibility of Gram Positive Cocci

Antimicrobial	*S. pneumoniae* (n = 85)			*S. aureus* (n = 31)		
	MIC (mg/L)			MIC (mg/L)		
	Range	50%	90%	Range	50%	90%
Gemifloxacin	≤0.02–0.06	0.03	0.06	0.03–1	0.06	0.06
Moxifloxacin	≤0.02–0.25	0.12	0.25	0.03–0.12	0.06	0.06
Trovafloxacin	≤0.02–0.12	0.06	0.12	≤0.02–0.06	0.03	0.03
Antimicrobial	*S. pneumoniae* (n = 85)			*S. aureus* (n = 31)		
	MIC (mg/L)			MIC (mg/L)		
	Range	50%	90%	Range	50%	90%
Grepafloxacin	0.03–0.5	0.25	0.25	0.06–0.25	0.12	0.12

Levofloxacin	0.12–2	1	1	0.12–0.5	0.25	0.25
Ofloxacin	0.25–4	2	2	0.25–1	0.5	0.5
Sparfloxacin	0.03–0.5	0.25	0.5	0.3–0.12	0.06	0.12
Ciprofloxacin	0.06–2	0.5	1	0.12–1	0.5	1
Amoxycillin	≤0.02–1	0.03	0.03	0.06–2	1	1
Amox/clav	≤0.02–1	≤0.02	0.03	0.03–2	1	1

Table 3. Susceptibility of *Haemophilus* spp.

Antimicrobial	*H. influenzae* (n = 45)			*H. parainfluenzae* (n = 10)		
	MIC (mg/L)			MIC (mg/L)		
	Range	50%	90%	Range	50%	90%
Gemifloxacin	≤0.02–0.03	≤0.02	≤0.02	≤0.02–0.12	0.06	0.12
Moxifloxacin	≤0.02–0.12	0.13	0.06	0.06–0.5	0.25	0.5
Trovafloxacin	≤0.02–0.06	≤0.02	0.03	≤0.02–0.12	0.03	0.12
Grepafloxacin	≤0.02–0.03	≤0.02	≤0.02	≤0.02–0.12	0.06	0.1
Levofloxacin	≤0.02–0.03	0.03	0.03	0.03–0.25	0.06	0.25
Ofloxacin	≤0.02–0.06	0.03	0.06	0.03–0.5	0.12	0.5
Sparfloxacin	0.03–1	0.25	0.25	0.12–1	0.5	1
Ciprofloxacin	≤0.02	≤0.02	≤0.02	≤0.02–0.06	0.03	0.06
Amoxycillin	0.06–64	0.25	2	0.03–1	0.06	1
Amox/clav	≤0.02–1	0.25	0.5	0.03–0.5	0.25	0.5

Table 4. Susceptibility of *Moraxella catarrhalis*

Antimicrobial	*M. catarrhalis* (n = 45)		
	MIC (mg/L)		
	Range	50%	90%
Gemifloxacin	≤0.02–0.03	≤0.02	≤0.02
Moxifloxacin	0.03–0.12	0.06	0.06
Trovafloxacin	≤0.02–0.06	≤0.02	0.03
Grepafloxacin	≤0.02–0.25	≤0.02	≤0.02
Levofloxacin	≤0.02–0.12	0.03	0.06
Ofloxacin	≤0.02–0.25	0.06	0.06
Sparfloxacin	≤0.02–1	≤0.02	0.5
Ciprofloxacin	≤0.02–0.25	0.03	0.03
Amoxycillin	≤0.02–16	1	8
Amox/clav	≤0.02–2	0.12	0.5

Table 5. Susceptibility of Anaerobic and Streptococcal Strains

Antimicrobial	Anaerobic strains (n = 22)*			*Streptococcus* spp.†		
	MIC (mg/L)			MIC (mg/L)		
	Range	50%	90%	Range	50%	90%
Gemifloxacin	0.03–0.25	0.12	0.25	≤0.02–0.5	0.12	0.5
Moxifloxacin	0.03–0.25	0.03	0.25	≤0.02–0.5	0.06	0.5
Trovafloxacin	0.06–4	1	4	≤0.02–0.5	0.06	0.5
Grepafloxacin	0.25–8	0.25	8	≤0.02–1	0.06	1
Levofloxacin	0.12–1	0.25	1	0.03–0.25	0.12	0.25
Ofloxacin	0.25–2	0.5	2	0.06–0.5	0.25	0.5
Sparfloxacin	0.25–16	4	16	≤0.02–0.5	0.03	0.5
Ciprofloxacin	0.06–1	0.5	1	≤0.02–0.12	0.12	0.12
Amoxycillin	0.25–8	0.25	8	0.03–≥256	2	4
Amox/clav	0.25–1	0.25	1	0.03–≥256	2	16

*Including 12 strains of *Bacteroides* spp., 7 strains of *Peptostreptococcus* spp. and 3 strains of *Bacteroides urealyticus*.

†Including 5 strains of *Enterobacteriaceae*, 6 strains of non-fermentative Gram negative bacilli, 2 strains of *Neisseria meningitidis* and 2 strains of beta-haemolytic *Streptococcus*.

The invention provides a method for modulating metabolism of maxillary sinus pathogenic bacteria. Skilled artisans can readily choose maxillary sinus pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by maxillary sinus pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with maxillary sinus pathogenic bacteria.

While a preferred object of the invention provides a method wherein said maxillary sinus pathogenic bacteria is selected from the group consisting of: a bacterial strain isolated from acute or chronic maxillary sinusitis; a maxillary sinus isolate of *Staphylococcus aureus*, *Streptococcus pneumoniae*, *Haemophilus* spp., *Moraxella catarrhalis*, an anaerobic strain or non-fermentative Gram negative bacilli, *Neisseria meningitidis*, β-haemolytic *Streptococcus*, *Haemophilus influenzae*, an *Enterobacteriaceae*, a non-fermentative Gram negative bacilli,

Streptococcus pneumoniae, *Streptococcus pyogenes*, a methicillin-resistant *Staphylococcus* spp., *Legionella pneumophila*, *Mycoplasma* spp. and *Chlamydia* spp., *Haemophilus influenzae*, *Haemophilus parainfluenzae*, *Peptostreptococcus*, *Bacteroides* spp., and *Bacteroides urealyticus*. Other maxillary sinus pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

(B) Methods of Using Fluoroquinolones Against Anaerobic Bacteria

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against anaerobic bacteria.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various anaerobic pathogens. Since anaerobic susceptibility testing is not routinely performed in most clinical laboratories, if it is performed at all, the clinician must rely on published studies to help guide both empirical therapy as well as specific therapy in situations that involve less commonly isolated or identified anaerobes or mixed infections at other sites. Most *in vitro* studies of gemifloxacin against anaerobic bacteria focus their attention on common intra-abdominal pathogens such as *Bacteroides fragilis* and *Clostridium perfringens* or lump the *anaerobes into large groups without speciation (Cormicon, et al., Antimicrob.* Agents Chemother., 41:204-211, 1997; Marco, et al., *J. Antimicrob. Chemother.*, 40:605-607, 1997). Consequently there is unmet medical need for data regarding certain species often recovered from respiratory and gynecological infections as well as less frequently identified components of mixed abdominal infections, as well as methods used for ther treatment of such pathogens.

The present invention was based, in part, on analyses showing that gemifloxacin compared favorably with trovafloxacin against the Gram-positive anaerobes tested as well as the other unusual isolates studied [see, for example, Table 6]. Cormicon and Jones (Cormicon, et al., *Antimicrob. Agents Chemother.*, 41:204-211, 1997) studied 10 strains of peptostreptococci and found an MIC $_{90}$ of 2 ug/ml for gemifloxacin which is in contrast to our study which included 45 strains of peptostreptococci from 4 species, all of which were susceptible to ⇔ 0.25 ug/ml of gemifloxacin. The reason for this discrepancy can not be accounted for by methodological variations since both studies used brucella agar and an agar dilution method. Marco, et al. (Marco et al. *J. Antimicrob. Chemother.*, 40:605-607, 1997)

studied 18 strains of peptostreptococci and also found an MIC $_{90}$ of 2 ug/ml [range, ⇔ 0.25-8 ug/ml] for gemifloxacin.

Differences in the susceptibility of different *Clostridium* species to gemifloxacin were apparent in assays described herein, with *C. clostridioforme* and *C. innocuum* being relatively susceptible while *C. difficile* was often resistant to gemifloxacin. In the current study, the ten *C. ramosum* isolates studied had an MIC$_{90}$ of 1 ug/ml while a prior study (Goldstein, et al., *Antimicrob. Agents Chemother*, Submitted) the MIC$_{90}$ for the 14 isolates studied was 8 ug/ml. With the exception of two strains, all isolates in these two studies were different and most came from blood cultures. The apparent disparity comes from the higher MICs of 3/14 strains in the prior study and highlights the problem of testing small numbers of isolates of a single species, which has been solved by the analyses underpinning certain embodiments of the current invention. Further, Cormicon and Jones (Cormicon, et al., *Antimicrob. Agents Chemother*., 41:204-211, 1997) studied ten clostridial isolates and found a maximum MIC of 2 ug/ml to gemifloxacin. Marco, et al. (Marco, et al., *J. Antimicrob. Chemother*. 40:605-607, 1997) reported all 19 unspecieted clostridial isolates they studied to be susceptible to ⇔ 2ug/ml.

The data presented herein shows that there is marked variation in susceptibility patterns of different anaerobic genera and species to trovafloxacin and gemifloxacin and that important clinical anaerobic isolates should have individual strain susceptibilities determined. It is difficult to predict susceptibility based on a grouping of several species in a less commonly encountered or identified genus, and this problem has been solved in the methods of the invention.

Gemifloxacin exhibited good activity against Gram-positive anaerobes, especially the four *Peptostreptococcus* species tested, as well as the *Porphyromonas* species tested.

As provided herein, activities of gemifloxacin and comparator antimicrobial agents were determined by an agar dilution method against 419 clinical strains of less commonly identified, though medically important, species of anaerobes. Gemifloxacin was generally more active than trovafloxacin against Gram-positive strains by one to two dilutions. Peptostreptococci [*P. asaccharolyticus*, *P. magnus*, *P. micros*, and *P. prevotii*] and *Porphyromonas* spp. [*P. asaccharolytica*, *P. canoris*, *P. gingivalis*, P. *macaccae*] were all susceptible to ⇔0.25 ug/ml of gemifloxacin. *Actinomyces israelii*, *Actinomyces odontolyticus*, *Clostridium innocuum*, *Clostridium clostridioforme*, *Anaerobiospirillum* spp., *Bacteroides tectum*, *B. ureolyticus*, *B. gracilis* [now *Campylobacter gracilis*], *Prevotella intermedia*, *Prevotella heparinolytica*, *Prevotella oris-buccae* group had MIC$_{90}$s of ⇔2 ↘g/ml.

Fusobacterium naviforme and F. necrophorum were also susceptible to ⇔2 ↘g/ml, while *F. varium* strains exhibited a bimodal pattern; the other *Fusobacterium* species, such as *F. ulcerans, F. russii,* as well as *Veillonella* spp., *Prevotella melaninogenica* group, *P. bivia, Clostridium difficile*, and *Bilophila wadsworthia* were relatively resistant to gemifloxacin [MIC_{90}s ⇩4 ug/ml].(See Table 6).

Table 6. In vitro activity [ug/ml] gemifloxacin, trovafloxacin, and other oral antimicrobial agents against unusual anaerobic pathogens.

Minimal Inhibitory Concentration

Organism & Agent (no. isolates) [A]	Range	50%	90%
Actinomyces odontolyticus [10]			
Gemifloxacin	1-2	2	2
Trovafloxacin	2-4	4	4
Penicillin G	0.125-0.25	0.125	.0125
Amoxicillin clavulanate	0.06-0.125	0.125	0.25
Clindamycin	⇔0.015-0.5	0.125	0.25
Erythromycin	⇔0.015-0.03	⇔0.015	0.03
Azithromycin	⇔0.015-0.06	0.03	0.06
Clarithromycin	⇔0.015	⇔0.015	⇔0.015
Metronidazole	4->32	16	32

Table 6. (Continued)

Actinomyces israelii [6]			
Gemifloxacin	0.5-2	1	
Trovafloxacin	0.5-2	1	
Penicillin G	⇔0.015-0.25	0.03	
Amoxicillin clavulanate	0.03-1	0.03	
Clindamycin	0.06-0.25	0.06	
Erythromycin	0.03	0.03	
Azithromycin	0.06	0.06	
Clarithromycin	⇔0.015	⇔0.015	
Metronidazole	1-32	4	
Anaerobiospirillum thomasii [13]			
Gemifloxacin	0.06-0.25	0.125	0.125
Trovafloxacin	0.06-0.5	0.125	0.25
Penicillin G	0.06-0.125	0.06	0.125
Amoxicillin clavulanate	0.125-0.25	0.125	0.25
Clindamycin	8->32	32	>32
Erythromycin	1-16	4	8
Azithromycin	0.125-1	0.5	1
Clarithromycin	2-32	4	16
Metronidazole	1-4	2	4
Anaerobiospirillum succiniciproducens [3			
Gemifloxacin	0.5-2	1	
Trovafloxacin	0.5-2	1	
Penicillin G	0.5-1	0.5	
Amoxicillin clavulanate	0.25-0.5	0.25	
Clindamycin	32	32	
Erythromycin	8-16	16	
Azithromycin	0.5-1	0.5	
Clarithromycin	8-32	32	
Metronidazole	4-8	8	
Bacteroides gracilis [11]			
Gemifloxacin	⇔0.015-1	⇔0.015	1
Trovofloxacin	⇔0.015-2	0.03	0.5
Penicillin G	⇔0.015-4	0.125	4
Amoxicillin clavulanate	0⇔.015-2	0.5	2
Clindamycin	0.03-8	0.25	2
Erythromycin	0.125-2	1	2
Azithromycin	0.06-0.5	0.125	0.5
Clarithromycin	0.25-2	1	1
Metronidazole	0.06>32	0.5	>32
Bacteroides tectum [22]			
Gemifloxacin	0.06-8	0.125	0.25
Trovafloxacin	0.03-0.125	0.06	0.125
Penicillin G	⇔0.015-32	0.03	16
Amoxicillin clavulanate	0.03-0.5	0.06	0.5
Clindamycin	⇔0.015—0.125	⇔0.015	⇔0.015
Erythromycin	0.25-1	0.5	0.5
Azithromycin	0.5-2	1	2
Clarithromycin	0.125	0.125	0.125
Metronidazole	0.125-2	0.5	0.5

Table 6. (Continued)

Bacteroides ureolyticus [17]			
Gemifloxacin	⇔0.015-2	⇔0.015	2
Trovafloxacin	⇔0.015-4	0.06	4
Penicillin G	⇔0.015-1	⇔0.015	0.25
Amoxicillin clavulanate	⇔0.015-1	⇔0.015	0.125
Clindamycin	0.03-0.5	0.06	0.25
Erythromycin	0.125-2	.025	2
Azithromycin	0.06-0.25	0.06	0.25
Clarithromycin	0.125-4	0.5	2
Metronidazole	0.06-2	0.25	1
Bilophila wadsworthia [16]			
Gemifloxacin	0.125->8	0.25	4
Trovofloxacin	0.125->8	0.5	>8
Penicillin G	2-16	4	8
Amoxicillin clavulanate	1-4	2	4
Clindamycin	0.25-2	0.5	2
Erythromycin	4-32	16	32
Azithromycin	1-16	4	16
Clarithromycin	4-32	16	32
Metronidazole	0.125	0.125	0.125
Clostridium clostridioforme [11]			
Gemifloxacin	0.5->8	0.5	1
Trovafloxacin	1-8	4	4
Penicillin G	0.5->32	1	16
Amoxicillin clavulanate	0.5-8	0.5	1
Clindamycin	⇔0.015-2	0.06	2
Erythromycin	0.25->32	16	>32
Azithromycin	0.125->32	16	>32
Clarithromycin	0.125->32	4	>32
Metronidazole	0.03-1	0.125	0.5
Clostridium difficile [14]			
Gemifloxacin	1->8	2	>8
Trovafloxacin	0.5->8	1	>8
Penicillin G	1-4	2	4
Amoxicillin clavulanate	0.5-1	1	1
Clindamycin	0.25->32	0.5	>32
Erythromycin	0.25>32	0.5	>32
Azithromycin	1->32	2	>32
Clarithromycin	0.125->32	0.5	>32
Metronidazole	0.25-1	0.5	0.5
Clostridium inocuum [11]			
Gemifloxacin	0.125->8	0.25	2
Trovafloxacin	0.25->8	0.5	8
Penicillin G	0.25->3	0.5	0.5
Amoxicillin clavulanate	0.5-2	0.5	0.5
Clindamycin	0.25->32	0.5	>32
Erythromyccin	0.5->32	>32	>32
Azithromycin	0.125->32	>32	>32
Clarithromycin	0.25->32	>32	>32
Metronidazole	0.5-2	0.5	1

Table 6. (Continued)

Clostridium ramosum [10]			
Gemifloxacin	0.125-2	0.25	1
Trovafloxcin	0.25-8	0.5	2
Pencillin G	0.06-1	0.06	1
Amoxicillin clavulanate	0.06-0.25	0.06	0.25
Clindamycin	0.25-4	2	2
Erythromycin	0.5->32	1	>32
Azithromycin	0.125->32	0.25	>32
Clarithromycin	0.25->32	0.5	>32
Metronidazole	1	1	1
Fusobacterium spp group 1 [19][B]			
Gemifloxacin	0.06-8	0.25	8
Trovafloxacin	0.25-4	0.5	4
Penicillin G	⇔0.015-16	⇔0.015	2
Amoxicillin clavulanate	⇔0.015-0.25	0.06	0.125
Clindamycin	⇔0.015-2	0.06	0.125
Erythromycin	1->32	8	32
Azithromycin	0.06-32	1	8
Clarithromycin	⇔0.015-32	8	32
Metronidazole	0.125-0.5	0.25	4
Fusobacterium spp. group 2 [12] [C]			
Gemifloxacin	0.125->8	4	4
Trovafloxacin	1->8	4	4
Penicillin G	⇔0.015->32	0.25	0.5
Amoxicillin clavulanate	0.125->4	1	2
Clindamycin	0.06-8	1	8
Erythromycin	8->32	>32	>32
Azithromycin	1->32	16	32
Clarithromycin	4->32	>32	>32
Metronidazole	0.125-1	0.5	1
Fusobacterium russii			
Gemifloxacin	0.5->8	>8	>8
Trovafloxacin	0.5-4	4	4
Penicillin G	⇔0.015-0.06	0.03	0.06
Amoxicillin clavulanate	⇔0.015-0.25	0.06	0.125
Clindamycin	⇔0.015-0.125	0.03	0.06
Erythromycin	1->32	4	>32
Azithromycin	0.03-32	0.25	32
Clarithromycin	2->32	4	>32
Metronidazole	⇔0.015-0.25	0.125	0.25

Table 6. (Continued)

Fusobacterium varium [17]			
Gemifloxacin	0.25->8	>8	>8
Trovafloxacin	0.5->8	4	>8
Penicillin G	0.03->32	0.5	8
Amoxicillin clavulanate	0.125-4	2	4
Clindamycin	0.06-16	4	16
Erythromycin	32->32	>32	>32
Azithromycin	2->32	32	>32
Clarithromycin	32->32	>32	>32
Metronidazole	0.125-4	1	2
Peptostreptococcus asaccharolyticus [11]			
Gemifloxacin	0.125-0.25	0.25	0.25
Trovafloxacin	0.5-2	1	1
Penicillin G	⇔0.015-1	0.03	0.25
Amoxicillin clavulanate	0.03-1	0.03	0.125
Clindamycin	⇔0.015->32	0.06	>32
Erythromycin	1->32	4	>32
Azithromycin	0.5->32	4	>32
Clarithromycin	0.5->32	2	>32
Metronidazole	0.125-2	0.5	1
Peptostreptococcus magnus [13]			
Gemifloxacin	0.030.03	0.03	0.06
Trovafloxacin	0.06-0.25	0.125	0.25
Penicillin G	⇔0.015-1	0.03	0.25
Amoxicillin clavulanate	0.03-1	0.03	0.125
Clindamycin	0.06-2	0.5	2
Erythromycin	1->32	4	>32
Azithromycin	2->32	4	>32
Clarithromycin	0.5->32	2	>32
Metronidazole	0.25-2	0.5	0.5
Peptostreptococcus micros [12]	0.06-0.125	0.06	0.06
Gemifloxacin			
Trovafloxacin	0.03-0.125	0.06	0.06
Penicillin G	⇔0.015-0.03	⇔0.015	0.03
Amoxicillin clavulanate	0.03-0.125	0.03	0.125
Erythromycin	0.5-1	0.5	0.5
Azithromycin	0.5-1	0.5	1
Clarithromycin	0.6	0.5	0.5
Clindamycin	0.06-0.125	0.125	0.125
Metronidazole	0.03-0.25	0.25	0.25
Peptostreptococcus prevotii [9]			
Gemifloxacin	0.06-0.25	0.125	-
Trovafloxacin	0.25-1	0.25	-
Penicillin G	0.03-0.06	0.03	-
Amoxicillin clavulanate	⇔0.015-0.125	0.03	-
Clindamycin	0.030-32	1	-
Erythromycin	0.03->32	>32	-
Azithromycin	0.06->32	32	-
Clarithromycin	⇔0.015->32	>32	-
Metronidazole	0.125-1	0.5	-

TABLE 6. (Continued)

Porphyromonas asaccharolyticus [11]			
Gemifloxacin	0.06-0.125	0.06	0.125
Trovafloxacin	0.03-0.25	0.25	0.25
Penicillin G	⇔0.015	⇔0.015	⇔0.015
Amoxicillin clavulanate	⇔0.015-0.03	⇔0.015	0.03
Clindamycin	⇔0.015->32	⇔0.015	>32
Erythromycin	0.03-32	0.03	32
Azithromycin	0.125->32	0.25	>32
Clarithromycin	⇔0.015->32	0.06	>32
Metronidazole	⇔0.015	⇔0.015	⇔0.015
Porphyromonas canoris [10]			
Gemifloxacin	0.06-0.25	0.25	0.25
Trovafloxacin	0.06-0.5	0.25	0.5
Penicillin G	⇔0.015-0.03	⇔0.015	⇔0.015
Amoxicillin clavulanate	⇔0.015-0.03	⇔0.015	0.03
Clindamycin	⇔0.015	⇔0.015	⇔0.015
Erythromycin	0.03-0.25	0.06	0.125
Azithromycin	0.125-0.5	0.25	0.25
Clarithromycin	0.06-0.125	0.06	0.125
Metronidazole	⇔0.015-0.5	0.25	0.25
Porphyromonas gingivalis [13]			
Gemifloxacin	⇔0.015-0.125	0.06	0.125
Trovafloxacin	0.03-0.06	0.06	0.06
Penicillin G	⇔0.015-0.06	⇔0.015	0.03
Amoxicillin clavulanate	⇔0.015-0.06	⇔0.015	0.06
Clindamycin	⇔0.015	⇔0.015	⇔0.015
Erythromycin	0.06-0.5	0.125	0.5
Azithromycin	0.125-1	0.25	0.5
Clarithromycin	0.06-0.125	0.06	0.125
Metronidazole	⇔0.015-0.03	⇔0.015	0.03
Porphyromonas macaccae [13]			
Gemifloxacin	0.03-0.125	0.06	0.125
Trovafloxacin	0.03-0.125	0.06	0.125
Penicillin G	⇔0.015-1	0.5	0.5
Amoxicillin clavulanate	⇔0.015-0.06	⇔0.015	⇔0.015
Clindamycin	⇔0.015-0.03	⇔0.015	⇔0.015
Erythromycin	0.06-0.25	0.125	0.25
Azithromycin	0.125-1	0.5	0.5
Clarithromycin	0.06-0.125	0.125	0.125
Metronidazole	⇔0.015-0.125	0.06	0.125
Porphyromonas spp. [11] D			
Gemifloxacin	0.06-0.125	0.06	0.125
Trovafloxacin	0.06-1	0.25	1
Penicillin G	⇔0.015-4	⇔0.015	⇔0.015
Amoxicillin clavulanate	⇔0.015-0.06	⇔0.015	⇔0.015
Clindamycin	⇔0.015	⇔0.015	⇔0.015
Erythromycin	⇔0.015-0.5	0.06	0.06
Azithromycin	0.125-1	0.25	0.5
Clarithromycin	0.06-0.125	0.06	0.125
Metronidazole	⇔0.015-0.25	0.03	0.125

Table 6. (Continued)

Prevotella bivia [21]			
Gemifloxacin	4->8	8	8
Trovafloxacin	1-4	2	2
Penicillin G	0.25-32	16	32
Amoxicillin clavulanate	0.06-4	0.5	4
Clindamycin	⇔0.015->32	⇔0.015	0.03
Erythromycin	0.06->32	1	2
Azithromycin	0.25->32	0.5	1
Clarithromycin	0.06->32	0.125	0.25
Metronidazole	0.5-4	2	4
Prevotella buccae-oris group [22] [E]			
Gemifloxacin	0.5-8	2	2
Trovafloxacin	0.25-4	1	2
Penicillin G	0.06->32	8	>32
Amoxicillin clavulanate	0.125-2	0.25	1
Erythromycin	0.5-8	1	2
Azithromycin	0.125-4	0.5	1
Clarithromycin	0.06-1	0.125	0.25
Clindamycin	⇔0.015-0.125	⇔0.015	0.03
Metronidazole	0.5-4	1	2
Prevotella heparinolytica [16]			
Gemifloxacin	0.25-0.5	0.5	0.5
Trovafloxacin	0.125-0.25	0.125	0.25
Penicillin G	0.06-0.25	0.06	0.125
Amoxicillin clavulanate	0.06-0.25	0.125	0.25
Clindamycin	⇔0.015	⇔0.015	⇔0.015
Erythromycin	0.25-0.5	0.25	0.25
Azithromycin	0.5-1	0.5	1
Clarithromycin	0.06-0.125	0.125	0.125
Metronidazole	0.06-1	0.5	1
Prevotella intermedia [11]			0.5
Gemifloxacin	0.06-1	0.25	
Trovafloxacin	0.06-1	0.5	1
Penicillin G	⇔0.015-16	0.03	4
Amoxicillin clavulanate	0.03-0.5	0.03	0.125
Clindamycin	⇔0.015-0.03	⇔0.015	⇔0.015
Erythromcyin	0.03-0.5	0.06	0.25
Azithromycin	0.03-1	0.125	0.5
Clarithromycin	⇔0.015-0.125	⇔0.015	0.125
Metronidazole	0.03-1	0.5	1
Prevotella melaninogenica [12]			
Gemifloxacin	0.125->8	1	8
Trovafloxacin	0.06-8	1	4
Penicillin G	⇔0.015-2	0.25	2
Amoxicillin clavulanate	0.03-16	2	4
Clindamycin	⇔0.015-32	⇔0.015	0.5
Erythromycin	0.06-32	1	8
Azithromycin	0.125->32	0.25	32
Clarithromycin	0.06-4	0.125	1
Metronidazole	0.125-4	0.5	1

Table 6. (Continued)

Prevotella denticola/loeschii group [6]			
Gemifloxacin	0.25-8	0.5	
Trovofloxacin	0.06-4	1	
Penicillin G	⇔0.015-32	4	
Amoxicillin clavulanate	0.03-0.5	0.06	
Clindamycin	⇔0.015-0.25	⇔0.015	
Erythromycin	0.125-16	0.25	
Azithromycin	0.06-16	0.5	
Clarithromycin	0.03-2	0.06	
Metronidazole	0.5-1	1	
Veillonella spp. [24]			
Gemifloxacin	0.03->8	1	8
Trovafloxacin	0.125->8	0.25	>8
Penicillin G	⇔0.015-8	1	4
Amoxicillin clavulanate	⇔0.015->4	0.5	2
Clindamycin	0.03->32	0.06	2
Erythromycin	1->32	16	>32
Azithromycin	0.125->32	4	>32
Clarithromycin	1->32	16	>32
Metronidazole	0.25-2	1	2

A MIC $_{50}$, MIC $_{90}$- Minimal inhibitory concentration for 50% and 90% of isolates tested, respectively

B - *Fusobacterium gonidiaformans*, 1; *Fusobacterium naviforme*, 8; *Fusobacterium necrophorum*, 8; *Fusobacterium nucleatum*, 1; *Fusobacterium nucleatum* ss *animalis*, 1.

C - *Fusobacterium mortiferum*, 2; *Fusobacterium necrogenes*, 3; *Fusobacterium ulcerans*, 7.

D - *Porphyromonas cangingivalis*, 4; *Porphyromonas cansulci*, 2; *Porphyromonas circumdentaria*, 2; *Porphyromonas levii*, 3.

E - *Prevotella buccae*, 20; *Prevotella oris*, 2.

The invention provides a method for modulating metabolism of anaerobic pathogenic bacteria. Skilled artisans can readily choose anaerobic pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by anaerobic pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with anaerobic pathogenic bacteria.

While a preferred object of the invention provides a method wherein said anaerobic pathogenic bacteria is selected from the group consisting of: selected from the group consisting of: a member of the genus *Peptostreptococci*, a *Peptostreptococci asaccharolyticus*, a

Peptostreptococci magnus, a *Peptostreptococci micros*, a *Peptostreptococci prevotii*, a member of the genus *Porphyromonas*, a *Porphyromonas asaccharolytica*, a *Porphyromonas canoris*, a *Porphyromonas gingivalis*, a *Porphyromonas macaccae*, a member of the genus *Actinomyces*, an *Actinomyces israelii*, an *Actinomyces odontolyticus*, a member of the genus *Clostridium*, a *Clostridium innocuum*, a *Clostridium clostridioforme*, a *Clostridium difficile*, a member of the genus *Anaerobiospirillum*, a member of the genus *Bacteroides*, a *Bacteroides tectum*, a *Bacteroides ureolyticus*, a *Bacteroides gracilis* (*Campylobacter gracilis*), a member of the genus *Prevotella*, a *Prevotella intermedia*, a *Prevotella heparinolytica*, a *Prevotella oris-buccae*, a *Prevotella bivia*, a *Prevotella melaninogenica*, a member of the genus *Fusobacterium*, a *Fusobacterium naviforme*, a *Fusobacterium necrophorum*, a *Fusobacterium varium*, a *Fusobacterium ulcerans*, a *Fusobacterium russii*, a member of the genus *Bilophila*, a *Bilophila wadsworthia*.

Other anaerobic pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

Preferred embodiments of the invention include, among other things, methods wherein said composition comprises gemifloxacin, or a pharmaceutically acceptable derivative thereof.

(C) Methods of Using Fluoroquinolones Against Atypical Upper Respiratory Tract Bacteria

The present invention provides, among other things, methods for using a composition comprising a gemifloxacin compound against atypical upper respiratory pathogenic bacteria, especially a member of the genus *Legionella*, a member of the genus, *Pseudomonas*, *Pseudomonas aeruginosa* strain, a *L. pneumophila* strain, a *L. pneumophila* serogroup 1, a *L. pneumophila* serogroup 2, a *L. pneumophila* serogroup 3, a *L. pneumophila* serogroup 4, a *L. pneumophila* serogroup 5, a *L. pneumophila* serogroup 6, a *L. pneumophila* serogroup 7, a *L. pneumophila* serogroup 8, a *L. dumoffii* strain, a *L. longbeacheae* strain, a L. micdadei strain, a L. oakridgensis strain, a *L. feelei* strain, a *L. anisa* strain, a *L. sainthelensi* strain, a *L. bozemanii* strain, a *L. gormanii* strain, a *L. wadsworthii* strain, a *L. jordanis* strain or a *L. gormanii* strain.

This invention was based, in part, on analyses evaluating the *in vitro* activity and postantibiotic effect (herein "PAE") of gemifloxacin compared with those of trovafloxacin, moxifloxacin, grepafloxacin, levofloxacin, ofloxacin, ciprofloxacin, azithromycin, clarithromycin, erythromycin and rifampicin against isolates of *Legionella pneumophila* and other *Legionella* spp. Test isolates included *L. pneumophila* serogroup 1–12 (204), *L. dumoffii*

(10), *L. micdadei* (10) and *L. longbeacheae* (7). The PAE was determined by exposing the isolates to the test antimicrobials for 1 hour at four times the minimum inhibitory concentration (herein "MIC"). The antimicrobial was removed by three consecutive centrifugations into fresh broth. The PAE was calculated by measuring bacterial growth kinetics in similar antimicrobial-free cultures. Rifampicin and trovafloxacin were the most active agents tested (MIC_{90} ≤0.008 mg/L). Gemifloxacin displayed high potency (MIC_{90} 0.016 mg/L) which was comparable to levofloxacin, grepafloxacin and moxifloxacin (MIC_{90} 0.016 mg/L) and more active than ciprofloxacin and ofloxacin (MIC_{90} 0.03 mg/L). Against *L. dumoffii* and *L. longbeachae*, gemifloxacin (MIC_{90} 0.06 mg/L) was as active than ciprofloxacin, ofloxacin, grepafloxacin and moxifloxacin. Against erythromycin-resistant *L. pneumophila*, gemifloxacin showed the longest PAE at 4.65 hours, compared with 4.18 hours for grepafloxacin, 3.38 hours for moxifloxacin and 2.83 hours for trovafloxacin. The gemifloxacin PAE was significantly superior to that of rifampicin (0.9 h), clarithromycin (1.9 h) and levofloxacin (2.59 h). Against erythromycin-susceptible *L. pneumophila* only gemifloxacin, moxifloxacin, levofloxacin, ofloxacin and ciprofloxacin had a PAE over 3 hours. For erythromycin-resistant *Legionella* spp. other than *L. pneumophila*, gemifloxacin, moxifloxacin, levofloxacin and ofloxacin had PAEs in excess of 3 hour, which was significantly longer than the PAE of ciprofloxacin grepafloxacin and erythromycin. The half-life for gemifloxacin and the data presented here indicate a significant PAE to support a once-daily administration of this agent for the treatment of *Legionella* infections, and this dosing is preferred in the methods of the invention.

The MIC range of gemifloxacin against *L. pneumophila* serogroups 1–9 was 0.008–0.06 mg/L (Tables 8 and 9). Gemifloxacin was 5–6-fold more active than erythromycin against *L. pneumophila* strains tested. Gemifloxacin activity against *L. pneumophila* strains was higher than ciprofloxacin and ofloxacin but similar to grepafloxacin and moxifloxacin.

L. pneumophila strains of serogroups 1–3 and 7–9 were more susceptible to gemifloxacin than *L. pneumophila* serogroups 4–6. Against the most frequent *L. pneumophila*, such as serogroup 1, gemifloxacin MIC_{90} (0.016 mg/L) was superior to azithromycin, clarithromycin, erythromycin, ofloxacin and ciprofloxacin.

Against *L. dumoffii* and *L. longbeacheae*, gemifloxacin, grepafloxacin and clarithromycin showed superior activity to azithromycin and erythromycin (Table 10). Against *L. micdadei*, gemifloxacin, ciprofloxacin, ofloxacin and moxifloxacin MICs were 5-fold more active than erythromycin.

Against erythromycin-resistant *L. pneumophila* only gemifloxacin, moxifloxacin and grepafloxacin displayed a mean PAE of >3 hours (Table 11). Clarithromycin, erythromycin

and rifampicin showed a PAE of <2 hours against these strains. Against erythromycin-resistant *Legionella* spp. other than *pneumophila*, gemifloxacin, grepafloxacin, levofloxacin, ofloxacin and rifampicin displayed a mean PAE of >3 hours, and erythromycin and clarithromycin of <2 hours.

Against erythromycin-susceptible *L. pneumophila*, gemifloxacin, moxifloxacin, ofloxacin and ciprofloxacin displayed a mean PAE of >3hours. Gemifloxacin and ofloxacin were the only quinolones showing a mean PAE of >2 hours against erythromycin-susceptible *Legionella* spp. other than *L. pneumophila*.

Gemifloxacin is an effective antimicrobial agent against most *Legionella* spp. and was significantly superior to the commonly used legionellosis therapy, erythromycin.

Against erythromycin-susceptible *L. pneumophila*, the mean PAE of gemifloxacin (3.49 hours) was ≥1 h longer than that of trovafloxacin, levofloxacin and clarithromycin.

Against erythromycin-susceptible *Legionella* spp. other than *pneumophila* the mean PAE of gemifloxacin (2.27 hours) was ≥1 h longer than that of trovafloxacin, moxifloxacin and clarithromycin.

Against erythromycin-resistant *L. pneumophila*, the mean PAE of gemifloxacin (4.65 hours) was significantly superior to the mean PAE of trovafloxacin, levofloxacin, ciprofloxacin, azithromycin, clarithromycin, erythromycin and rifampicin. A difference in mean PAE was also noted between gemifloxacin and trovafloxacin against *Legionella* spp. other than *L. pneumophila*.

The results of this study indicate that gemifloxacin should be a promising agent for the treatment of lower respiratory tract infections caused by *Legionella* spp.

Table 7. *Legionella* Strains Tested

Microrganism	No. of strains tested
L. pneumophila	204*
L. micdadei	10
L. dumoffii	10
L. longbeacheae	7
Others†	7

*10 different serogroups

†*oakridgensis, feelei, anisa, sainthelensi, bozemanii, gormanii, wadsworthii*

Table 8. Susceptibility of *Legionella pneumophila* Serogroups 1–4

Antimicrobial	*L. pneumophila* serogroup 1 (n = 85) MIC (mg/L)			*L. pneumophila* serogroup 2 (n = 17) MIC (mg/L)			*L. pneumophila* serogroup 3 (n = 15) MIC (mg/L)			*L. pneumophila* serogroup 4 (n = 26) MIC (mg/L)		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
Gemifloxacin	0.008–0.06	0.016	≤0.004	0.008–0.016	0.008	0.016	0.008–0.016	0.016	0.016	0.008–0.03	0.016	0.03
Trovafloxacin	≤0.004–0.016	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004
Moxifloxacin	≤0.004–0.03	0.016	0.016	≤0.004–0.016	0.008	0.008	≤0.004–0.016	0.008	0.016	≤0.004–0.016	0.016	0.016
Grepafloxacin	≤0.004–0.06	0.016	0.016	≤0.004–0.03	0.008	0.016	≤0.004–0.016	0.008	0.016	0.008–0.016	0.008	0.016
Levofloxacin	≤0.004–0.016	0.016	0.016	≤0.004–0.016	0.008	0.008	0.008–0.016	0.008	0.016	0.004–0.016	0.016	0.016
Ofloxacin	0.008–0.03	0.03	0.03	0.008–0.03	0.016	0.03	0.016–0.03	0.016	0.03	0.008–0.03	0.03	0.03
Ciprofloxacin	0.016–0.25	0.03	0.03	≤0.004–0.03	0.016	0.016	≤0.004–0.03	0.03	0.03	0.016–0.12	0.03	0.06
Azithromycin	0.008–1.0	0.06	0.5	0.008–0.12	0.06	0.12	0.016–0.25	0.12	0.25	0.008–0.25	0.12	0.12
Clarithromycin	≤0.004–0.12	0.06	0.06	≤0.004–0.06	0.03	0.06	0.016–0.06	0.03	0.06	0.004–0.06	0.03	0.06
Erythromycin	0.03–1.0	0.25	1.0	0.008–0.5	0.25	0.25	0.06–0.5	0.25	0.5	0.016–0.5	0.5	0.5
Rifampicin	≤0.004–0.008	≤0.004	0.008	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004–0.008	≤0.004	≤0.004

Table 9. Susceptibility of *L. pneumophila* Serogroups 5–12

Antimicrobial	*L. pneumophila* serogroup 5 (n = 15)			*L. pneumophila* serogroup 6 (n = 40)			*L. pneumophila* serogroup 7 (n = 2)			*L. pneumophila* serogroups 8, 9 and 12 (n = 4)		
	MIC (mg/L)			MIC (mg/L)			MIC (mg/L)			MIC (mg/L)		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
Gemifloxacin	0.03–0.06	0.03	0.03	0.008–0.03	0.016	0.03	0.008–0.016	0.008	0.016	0.016	0.016	0.016
Trovafloxacin	≤0.004–0.008	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004
Moxifloxacin	≤0.004–0.03	0.016	0.016	≤0.004–0.016	0.008	0.016	≤0.004–0.016	≤0.004	0.016	0.016	0.016	0.016
Grepafloxacin	≤0.004–0.003	0.016	0.03	≤0.004–0.016	0.008	0.016	≤0.004–0.008	≤0.004	0.008	0.008	0.008	0.008
Levofloxacin	≤0.004–0.016	0.008	0.016	0.008–0.016	0.008	0.016	0.008–0.016	0.008	0.016	0.008–0.016	0.008	0.016
Ofloxacin	0.008–0.03	0.016	0.03	0.008–0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Ciprofloxacin	0.016–0.06	0.03	0.03	≤0.004–0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Azithromycin	0.008–0.5	0.03	0.25	0.016–0.25	0.06	0.12	0.06	0.06	0.06	0.06	0.06	0.06
Clarithromycin	0.03–0.06	0.03	0.06	≤0.004–0.06	0.016	0.06	0.016–0.06	0.016	0.06	0.06	0.06	0.06
Erythromycin	0.06–1.0	0.25	0.5	0.008–0.25	0.12	0.25	0.12–0.5	0.12	0.5	0.25	0.25	0.25
Rifampicinl	≤0.004	≤0.004	≤0.004	≤0.004–0.008	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004

Table 10. Susceptibility of *Legionella* Other Than *pneumophila*

Antimicrobial	*L. dumoffii* (n = 10)			*L. micdadei* (n = 10)			*L. longbeacheae* (n = 7)			Other *Legionella* spp. (n = 7)*		
	MIC (mg/L)			MIC (mg/L)			MIC (mg/L)			MIC (mg/L)		
	Range	50%	90%	Range	50%	90%	Range	50%	90%	Range	50%	90%
Gemifloxacin	0.06	0.06	0.06	0.008–0.03	0.016	0.03	0.016–0.06	0.06	0.06	0.016–0.06	0.03	0.06
Trovafloxacin	≤0.004–0.008	0.008	0.008	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004	≤0.004
Moxifloxacin	0.008–0.03	0.03	0.03	0.008–0.03	0.016	0.03	0.008–0.03	0.016	0.03	0.008–0.03	0.008	0.03
Grepafloxacin	0.06	0.06	0.06	≤0.004–0.016	0.008	0.016	≤0.004–0.06	0.03	0.06	≤0.004–0.03	0.03	0.03
Levofloxacin	0.016	0.016	0.016	0.008–0.016	0.016	0.016	0.008–0.016	0.016	0.016	0.008–0.06	0.016	0.016
Ofloxacin	0.03	0.03	0.03	0.03	0.03	0.03	0.016–0.03	0.03	0.03	≤0.004–0.06	0.016	0.06
Ciprofloxacin	0.016–0.03	0.016	0.03	0.016–0.03	0.016	0.03	≤0.004–0.03	0.016	0.03	≤0.004–0.03	0.016	0.03
Azithromycin	0.12–0.25	0.12	0.25	0.016–0.25	0.25	0.25	0.016–0.25	0.12	0.25	0.016–0.5	0.12	0.5
Clarithromycin	0.03–0.06	0.03	0.06	0.03–0.12	0.06	0.06	0.008–0.06	0.06	0.06	≤0.004–0.12	0.03	0.12
Erythromycin	0.25–0.5	0.25	0.5	0.25–1	0.5	1	0.008–0.5	0.25	0.5	0.016–1	0.5	1
Rifampicin	≤0.004–0.03	0.008	0.016	0.008	0.008	0.008	≤0.004–0.06	≤0.004	0.06	≤0.004–0.008	≤0.004	0.008

*Includes one isolates of *L. bozemanii, L. feelei, L. jordanis, L. gormanii, L. oakridgensis, L. sainthelensi* and *L. wadsworthii*.

Table 11. Mean PAE of Antimicrobials Against Erythromycin-resistant and -susceptible Strains of *Legionella*

Antimicrobial (4xMIC)	Mean PAE (h)*			
	Erythromycin-resistant strains		Erythromycin-susceptible strains	
	L. pneumophila (n = 7)	*Legionella* spp.† (n = 9)	*L. pneumophila* (n = 15)	*Legionella* spp.** (n = 13)
Gemifloxacin	4.65 ± 3	3.34±2	3.49±3	2.27±2
Trovafloxacin	2.83 ± 2	2.25±2	1.71±1	1.22±1
Moxifloxacin	3.38±2	2.02±1	3.59±3	1.18±2
Grepafloxacin	4.18±3	3.67±1	2.62±3	1.67±1
Levofloxacin	2.59±2	3.24±1	2.14±2	1.35±1
Ofloxacin	2.99±1	4.13±2	3.53±3	3.04±2
Ciprofloxacin	2.86±2	2.13±3	3.61±2	1.86±2
Azithromycin	2.16±1	2.13±1	2.91±3	1.86±2
Clarithromycin	1.90±1	1.60±2	0.72±2	0.98±2
Erythromycin	0.90±1	0.44±1	0.93±1	2.06±2
Rifampicin	0.93±4	5.6±3	2.86±5	3.09±4

***Means are given ± SD**
†*L. micdadei* (n=1), *L. dumofii* (n=3), *L. bozemanii* (n=1), *L. wadsworthii* (n=1), *L. jordanis* (n=1), *L. longbeacheae* (n=2)
*****L. micdadei* (n=4), *L. dumofii* (n=5), *L. bozemanii* (n=1), *L. gormanii* (n=1), *L. jordanis* (n=1), *L. longbeacheae* (n=1)**

The invention provides a method for modulating metabolism of atypical upper respiratory pathogenic bacteria. Skilled artisans can readily choose atypical upper respiratory pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by atypical upper respiratory pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with atypical upper respiratory pathogenic bacteria.

While a preferred object of the invention provides a method wherein said atypical upper respiratory pathogenic bacteria is selected from the group consisting of: a member of the genus *Legionella,* a member of the genus, *Pseudomonas, Pseudomonas aeruginosa* strain, a *L. pneumophila* strain, a *L. pneumophila* serogroup 1, a *L. pneumophila* serogroup 2, a *L. pneumophila* serogroup 3, a *L. pneumophila* serogroup 4, a *L. pneumophila* serogroup 5, a *L. pneumophila* serogroup 6, a *L. pneumophila* serogroup 7, a *L. pneumophila* serogroup 8, a *L. dumoffii* strain, a *L. longbeacheae* strain, a L. micdadei strain, a L. oakridgensis strain, a *L. feelei* strain, a *L. anisa* strain, a *L. sainthelensi* strain, a *L. bozemanii* strain, a *L. gormanii* strain, a *L. wadsworthii* strain, a *L. jordanis*; strain and a *L. gormanii* strain.Other atypical upper respiratory pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

(D) Methods of Using Fluoroquinolones Against Mycoplasma Bacteria

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against a range of pathogenic bacteria.

This invention was based, in part, on analyses evaluating the *in vitro* activity of a gemifloxacin compound, as well as other new quinolones and macrolides using low-passaged clinical isolates and type strains of *Mycoplasma* species commonly found in the respiratory and urogenital tract of humans. Organisms used in the analyses included *Mycoplasma pneumoniae* (MPN), *M. hominis* (Mh), *M. fermentans* (Mf), *M. genitalium* (Mg), *M. penetrans* (Mp) and *Ureaplasma urealyticum* (Uu). Minimum Inhibitory

Concentrations (MICs) were determined using a micro-broth dilution method. Assays for *Ureaplasma urealyticum* were performed in 10B media and all other mycoplasma assays were carried out in SP4 medium. Comparator drugs, to which gemifloxacin was compared, as well as also being useful in the methods of the invention, include levofloxacin (Lev), trovafloxacin (Tro), grepafloxacin (Gre), azithromycin (Azi), clarithromycin (Cla), tetracycline (Tet) and clindamycin (Cli). The results of these MIC assays are shown in Table 12.

TABLE 12

	MIC 90 (ug/ml)							
Isolates (number)	**Gem**	**Lev**	**Trov**	**Grep**	**Azith**	**Clar**	**Tet**	**Clin**
MPN (103)	0.125	0.5	0.25	0.125	≤0.008	≤0.008	0.25	-
Mh (49)	≤0.008	0.25	0.031	0.031	-	-	32	≤0.008
Mf (19)	≤0.008	0.031	0.016	0.016	2	64	0.063	0.031
Uu (99)	0.25	1	0.125	1	4	0.063	1	-
MICs for Mg (2)	0.063	1	0.063	0.125	≤0.008	≤0.008	0.125	0.25
	0.063	0.5	0.063	0.125	≤0.008	≤0.008	0.063	0.25
MICs for Mp (1)	≤0.008	0.031	≤0.008	0.016	≤0.008	≤0.008	0.125	≤0.008

Depending on the species tested, gemifloxacin had variable results when compared to the macrolides. Gemifloxacin was equally as active or more active *in vitro* when compared to tetracycline, clindamycin and the other quinolones.

The invention provides a method for modulating metabolism of pathogenic Mycoplasma bacteria. Skilled artisans can readily choose pathogenic Mycoplasma bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by pathogenic Mycoplasma bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with pathogenic Mycoplasma bacteria.

While a preferred object of the invention provides a method wherein said pathogenic Mycoplasma bacteria is selected from the group consisting of: *Mycoplasma pneumoniae*, *M. hominis*, *M. fermentans*, *M. genitalium*, *M. penetrans* and *Ureaplasma urealyticum*. Other pathogenic Mycoplasma bacteria may also be included in the methods.

The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

(E) Methods of Using Fluoroquinolones Against Antiobiotic-Resistant Bacteria

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against a number of antibiotic compounds.

Previous studies have shown gemifloxacin to be 32 to 64 fold more active than ciprofloxacin, ofloxacin, sparfloxacin and trovafloxacin against methicillin-susceptible and -resistant Staphylococcus aureus, methicillin-resistant Staphylococcus epidermidis and S.pneumoniae. Gemifloxacin was also highly active against most members of the family Enterobacteriaceae, with activity was more potent than those of sparfloxacin and ofloxacin and comparable to that of ciprofloxacin. Gemifloxacin was the most active agent against Gram-positive species resistant to other quinolones and glycopeptides. Gemifloxacin has limited activity against anaerobes (Cormican, et al., *Antimicrob. Agents Chemother.* 41:204-211. 1997; Hohl, et al., *Clin. Microbiol. Infect.* 4:280-284, 1998; Oh, et al., *Antimicrob. Agents Chemother.* 40:1564-1568, 1996).

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various pneumococcal pathogens. In these analyses, gemifloxacin gave the lowest quinolone MICs against all pneumococcal strains tested followed by trovafloxacin, grepafloxacin, sparfloxacin, levofloxacin and ciprofloxacin. MICs were similar to those described previously (Cormican, et al., *Antimicrob. Agents Chemother.* 41:204-211. 1997; Hohl, et al., *Clin. Microbiol. Infect.* 4:280-284, 1998; Oh, et al., *Antimicrob. Agents Chemother.* 40:1564-1568, 1996). Additionally, gemifloxacin gave significantly lower MICs against highly quinolone resistant pneumococci, irrespective of quinolone resistance mechanism. This was the case in double mutants with mutations in both parC and gyrA, strains which have previously been shown to be highly resistant to other quinolones, as well as for strains with an efflux mechanism (Brenwald, et al., *Antimicrob. Agents Chemother.* 42:2032-2035, 1998; and Pan et al., *Antimicrob. Agents Chemother.* 40:2321-2326, 1996). MICs of non-quinolone agents were siomilar to those described previously (M.R. Jacobs, *Clin. Infect. Dis.* 15:119-127, 1992; Jacobs, et al., *Rev. Med. Microbiol.* 6:77-93, 1995; Pankuch, et al., *J. Antimicrob. Chemother.* 35:883-888, 1995).

Gemifloxacin also showed good killing against the 12 strains tested, including the two quinolone resistant strains. At ≤0.5 μg/ml, gemifloxacin was bactericidal against all 12 strains.

Killing rates relative to MICs were similar to those of other quinolones, with significant killing occurring earlier than ß-lactams and macrolides. Kill kinetics of quinolone and non-quinolone compounds in the analyses described herein were similar to those described previously (Pankuch, et al., *Antimicrob. Agents Chemother.* 38:2065-2072, 1994; Pankuch et al., *Antimicrob. Agents Chemother.* 40:1653-1656, 1996; and Visalli, et al., *Antimicrob. Agents Chemother.* 40:362-366, 1996). Gemifloxacin also gave, together with the other quinolones tested, significant PAEs against all 6 strains tested, including the one quinolone resistant strain. The higher ciprofloxacin PAE at both exposure concentrations is of no significance, because, with an MIC of 32 μg/ml, 5 x and 10 x MIC are not clinically achievable with this strain. PAE values for quinolones and macrolides were similar to those described previously (Fuursted, et al., *Antimicrob. Agents Chemother.* 41:781-784, 1997; Licata, et al., *Antimicrob. Agents Chemother.* 41:950-955, 1997; Spangler, et al., *Antimicrob. Agents Chemother.* 41:2173-2176, 1997; and Spangler, et al., *Antimicrob. Agents Chemother.* 42:1253-1255, 1998). It is generally accepted that quinolones have similar PAEs against pneumococci.

In summary, gemifloxacin was the most potent quinolone tested by MIC and time-kill against both quinolone susceptible and resistant pneumococci and, similar to other quinolones, gave PAEs against quinolone susceptible strains. The incidence of quinolone resistant pneumococci is currently very low. However, this situation may change with the introduction of broad-spectrum quinolones into clinical practice, and in particular in the pediatric population, leading to selection of quinolone resistant strains (Davies, et al., *Antimicrob. Agents Chemother.* 43:1177-1182, 1999). Gemifloxacin is a promising new antipneumococcal agent against pneumococci, irrespective of their susceptibility to quinolones and other agents. Clinical studies will be necessary in order to validate this hypothesis.

Results of agar dilution MIC testing of the 207 strains with ciprofloxacin MICs ≤4.0 μg/ml are presented in Table 13. $MIC_{50/90}$ values (μg/ml) were as follows: gemifloxacin, 0.03/0.06; ciprofloxacin, 1.0/4.0; levofloxacin, 1.0/2.0; sparfloxacin, 0.5/0.5; grepafloxacin, 0.125/0.5; trovafloxacin, 0.125/0.25; amoxicillin, 0.016/0.06 (penicillin susceptible), 0.125/1.0 (penicillin intermediate), 2.0/4.0 (penicillin resistant); cefuroxime, 0.03/0.25 (penicillin susceptible), 0.5/2.0 (penicillin intermediate), 8.0/16.0 (penicillin resistant); azithromycin, 0.125/0.5 (penicillin susceptible), 0.125/>128.0 (penicillin intermediate), 4.0/>128.0 (penicillin resistant); clarithromycin, 0.03/0.06 (penicillin susceptible), 0.03/32.0 (penicillin intermediate), 2.0/>128.0 (penicillin resistant). Against 28 strains with ciprofloxacin MICs ≥8 μg/ml, gemifloxacin had the lowest MICs (0.03-1.0 μg/ml, MIC_{90} 0.5

μg/ml), compared with MICs ranging between 0.25 to >32.0 μg/ml)(MIC_{90}s 4.0->32.0 μg/ml) for the other quinolones, with trovafloxacin, grepafloxacin, sparfloxacin and levofloxacin, in ascending order, giving the next lowest MICs (Table 14). Mechanisms of quinolone resistance are presented in Tables 15 and 16. As can be seen, quinolone resistance was associated with mutations in the quinolone resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB. Mutations in ParC were at S79-F or Y, D83-N, R95-C, or K137-N. Mutations in gyrA were at S83-A, C, F, or Y; E87-K; or S116-G. Twenty one strains had a mutation in parE at D435-N or I460-V. Only two strains had a mutation in gyrB at D435-N or E474-K. Twenty strains had a total of three or four mutations in the QRDRs or parC, gyrA, parE, and gyrB (Table 15). Amongst these 20 strains all were resistant to ciprofloxacin (MICs >8 μg/ml), levofloxacin (MICs >4 μg/ml), and sparfloxacin (MICs >1 μg/ml); 19 were resistant to grepafloxacin (MICs >1 μg/ml); and 10 were resistant to trovafloxacin (MICs >2 μg/ml), yet gemifloxacin MICs were < 0.5 μg/ml in 18 of the strains (Table 14).

In the presence of reserpine 23 strains had lower ciprofloxacin MICs (2-16 x); 13 strains had lower gemifloxacin MICs (2-4 x); 7 strains had lower levofloxacin MICs (2-4 x); 3 strains had lower grepafloxacin MICs (2 x); and one strain had lower sparfloxacin MICs (2 x), suggesting that an efflux mechanism contributed to the raised MICs in some cases (Table 16).

Microbroth dilution MIC results of the 12 strains tested by time-kill are presented in Table 17. Microdilution MICs were all within one dilution of agar MICs. For the two quinolone resistant strains (both penicillin susceptible), gemifloxacin microbroth MICs were 0.5 and 0.25 μg/ml, respectively. Time-kill results (Table 18) showed that levofloxacin at the MIC, gemifloxacin and sparfloxacin at 2 x MIC and ciprofloxacin, grepafloxacin and trovafloxacin at 4 x MIC, were bactericidal after 24 h. Various degrees of 90% and 99% killing by all quinolones was detected after 3 h. Gemifloxacin and trovafloxacin were both bactericidal at the microbroth MIC for the two quinolone resistant pneumococcal strains. Gemifloxacin was uniformly bactericidal after 24 h at ≤0.5 μg/ml. Amoxicillin, at 2 x MIC and cefuroxime at 4 x MIC, were bactericidal after 24 h, with some degree of killing at earlier time periods. By contrast, macrolides gave slower killing against the 7 susceptible strains tested, with 99.9% killing of all strains at 2-4 x MIC after 24 hours.

For the five quinolone susceptible strains tested for PAE, MICs were similar to those obtained by microdilution, with gemifloxacin having MICs of 0.25 μg/ml against the quinolone resistant strain (MICs of other quinolones 4-32 μg/ml). PAEs (h)(10 x MIC) for the 5 quinolone susceptible strains ranged between 0.4-1.6 for gemifloxacin; 0.5-1.5 h for ciprofloxacin (except for the quinolone resistant strain which gave a ciprofloxacin PAE of

6.3); 0.9-2.3 (levofloxacin); 0.3-2.0 (sparfloxacin); 0.3-2.6 (grepafloxacin); 1.3-3.0 (trovafloxacin). At 5 x MIC, PAEs (h) for the quinolone resistant strain were 0.9 (gemifloxacin); 3.7 (ciprofloxacin); 1.3 (levofloxacin); 1.5 (sparfloxacin); 1.5 (grepafloxacin); 1.3 (trovafloxacin). PAEs for non-quinolone compounds (10 x MIC) ranged between 0.3-5.8 (amoxicillin); 0.8-2.9 (cefuroxime); 1.3-3.0 (azithromycin); 1.8-4.5 (clarithromycin).

TABLE 13. Agar dilution MICs (μg/ml) of 207 quinolone susceptible strains[a]

Drug	MIC range	MIC_{50}	MIC_{90}
Penicillin			
Penicillin S	≤0.008-0.06	0.016	0.03
Penicillin I	0.125-1.0	0.25	1.0
Penicillin R	2.0-16.0	4.0	4.0
Gemifloxacin			
Penicillin S	≤0.008-0.125	0.03	0.03
Penicillin I	≤0.008-0.25	0.03	0.06
Penicillin R	0.004-0.125	0.03	0.06
Ciprofloxacin			
Penicillin S	0.25-4.0	1.0	2.0
Penicillin I	0.25-4.0	1.0	2.0
Penicillin R	0.5-4.0	1.0	4.0
Levofloxacin			
Penicillin S	0.125-4.0	1.0	2.0
Penicillin I	0.5-4.0	1.0	2.0
Penicillin R	1.0-2.0	1.0	2.0
Sparfloxacin			
Penicillin S	≤0.03-1.0	0.5	1.0
Penicillin I	0.06-2.0	0.5	0.5
Penicillin R	0.06-1.0	0.5	0.5
Grepafloxacin			
Penicillin S	≤0.03-1.0	0.125	0.5
Penicillin I	≤0.03-0.5	0.125	0.5
Penicillin R	≤0.03-0.5	0.25	0.5
Trovafloxacin			
Penicillin S	0.03-0.5	0.125	0.25
Penicillin I	0.016-1.0	0.125	0.25
Penicillin R	0.03-0.25	0.125	0.25
Amoxicillin			
Penicillin S	≤0.008-0.25	0.016	0.06
Penicillin I	0.016-4.0	0.125	1.0
Penicillin R	0.5-8.0	2.0	4.0
Cefuroxime			
Penicillin S	≤0.008-2.0	0.03	0.25
Penicillin I	0.125-8.0	0.5	2.0
Penicillin R	0.5-32.0	8.0	16.0

TABLE 13. (Continued)

Azithromycin			
Penicillin S	≤0.008->128.0	0.125	0.5
Penicillin I	≤0.008->128.0	0.125	>128.0
Penicillin R	0.03->128.0	4.0	>128.0
Clarithromycin			
Penicillin S	≤0.008->128.0	0.03	0.06
Penicillin I	≤0.008->128.0	0.03	32.0
Penicillin R	0.008->128.0	2.0	>128.0

[a]Ciprofloxacin MICs ≤4.0 μg/ml.

TABLE 14. Quinolone agar dilution MICs (μg/ml) of 28 ciprofloxacin resistant strains[a]

Quinolone	MIC range	MIC_{50}	MIC_{90}
Gemifloxacin	0.3-1.0	0.25	0.5
Ciprofloxacin	8.0->32.0	16.0	>32.0
Levofloxacin	4.0->32.0	16.0	>32.0
Sparfloxacin	0.25->32.0	8.0	16.0
Grepafloxacin	0.5-16.0	4.0	8.0
Trovafloxacin	0.25-8.0	1.0	4.0

[a]Ciprofloxacin MICs ≥8.0 μg/ml.

TABLE 15. Correlation of quinolone MIC (μg/ml) and mutation in quinolone resistant strains.

Strain	Gemifloxacin[a]	Ciprofloxacin[a]	Levofloxacin[a]	Sparfloxacin[a]	Grepafloxacin[a]	Trovafloxacin[a]	Mutation			
							ParC	ParE	GyrA	GyrB
1	0.03	16	8	4	4	2	S79-F	I460-V	S83-F	None
2	0.06	8	4	0.5	0.5	0.25	S79-Y	I460-V	None	None
3	0.06	8	4	1	0.5	0.25	D83-N	I460-V	S83-F	None
4	0.06	8	4	1	1	0.25	S79-F	I460-V	S83-F	None
5	0.125	8	8	1	1	0.25	R95-C	D435-N	S83-F	None
6	0.125	8	8	8	2	2	S79-Y	I460-V	E87-K	None
7	0.125	8	8	1	1	0.5	None	I460-V	None	None
8	0.125	8	8	1	1	0.5	S79-Y	None	None	None
9	0.125	8	8	2	1	1	S79-Y	None	None	None

TABLE 15. (Continued)

10	0.125	8	8	4	2	1	S79-F	I460-V	S83-C	None
11	0.125	8	8	4	4	1	S79-F	I460-V	S83-F	None
12	0.125	>32	16	1	4	1	S79-F	I460-V	None	D435-N
13	0.25	16	8	8	2	1	S79-F	I460-V	None	E474-K
14	0.25	16	16	8	4	1	S79-F	I460-V	S83-F	None
15	0.25	16	16	8	4	1	79-F	I460-V	S83-F	None
16	0.25	16	16	8	4	2	S79-F	I460-V	S83-F	None
17	0.25	16	16	8	4	2	D83-N	None	S83-F	None
18	0.25	16	16	8	4	2	S79-F	I460-V	S83-F	None
19	0.25	16	16	8	4	2	S79-F	I460-V	S83-F	None
20	0.25	32	16	8	4	2	S79-F	I460-V	E87-K	None

TABLE 15. (Continued)

21	0.25	32	16	8	4	1	S79-F	I460-V	S83-F	None
22	0.25	32	16	8	4	2	S79-F	I460-V	S83-Y	None
23	0.25	32	16	8	8	2	S79-Y	None	S83-A	None
24	0.5	32	32	16	8	4	S79-F	I460-V	S83-F	None
25	0.5	32	32	16	8	4		None	S83-F	None
26	0.5	>32	>32	>32	8	4	S79-F	None	S83-Y	None
27	1	>32	>32	>32	16	8	S79-Y	I460-V	S83-F	None
28	1	>32	>32	>32	16	8		None	S83-F S116-G	None

[a]MIC (μg/ml).

TABLE 16. Efflux mechanisms in quinolone resistant pneumococci

Strain	Gemifloxacin	Ciprofloxacin	Levofloxacin	Sparfloxacin	Grepafloxacin	Trovafloxacin
1	2 X[a]	2 X	-	-	-	-
2	-	8 x	-	-	-	-
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	2 X	4 X	-	2 X	2 X	-
6	-	2 X	-	-	-	-
7	2 X	4 X	2 X	-	-	-
8	2 X	2 X	-	-	-	-
9	4 X	8 X	-	-	-	-
10	-	2 X	-	-	-	-
11	2 X	16 X	4 X	-	-	-
12	2 X	4 X	2 X	-	2 X	-
13	-	2 X	-	-	-	-
14	-	-	-	-	-	-
15	-	-	-	-	-	-
16	2 X	4 X	-	-	-	-

TABLE 16. (Continued)

Strain	Gemifloxacin	Ciprofloxacin	Levofloxacin	Sparfloxacin	Grepafloxacin	Trovafloxacin
17	2 X	4 X	-	-	-	-
18	-	2 X	-	-	-	-
19	-	2 X	-	-	-	-
20	-	2 X	-	-	-	-
21	-	2 X	-	-	-	-
22	-	2 X	-	-	-	-
23	2 X	8 X	2 X	-	-	-
24	-	2 x	-	-	-	-
25	2 x	4 x	2 x	-	-	-
26	2 x	4 x	2 x	-	-	-
27	-	-	-	-	-	-
28	4 x	8 x	4 x	-	2 X	-

TABLE 17. Microdilution MICs of 12 strains tested by time-kill

Drug	1 (S)[a]	2 (S)	3 (S)[b]	4 (S)[b]	5 (I)	6 (I)	7 (I)	8 (I)	9 (R)	10 (I)	11 (R)	12 (R)
Penicillin G	0.06	0.03	0.016	0.016	0.25	0.25	1	0.5	4	2	4	4
Gemifloxacin	0.016	0.016	0.5	0.25	0.03	0.016	0.016	0.016	0.03	0.016	0.016	0.03
Ciprofloxacin	1	0.5	32	32	2	1	4	0.5	1	1	2	1
Levofloxacin	2	1	32	32	1	2	1	1	2	2	1	2
Sparfloxacin	0.125	0.25	32	16	0.5	0.25	0.25	0.25	0.5	0.25	0.25	0.5
Grepafloxacin	0.06	0.06	16	8	0.125	0.125	0.125	0.125	0.25	0.125	0.125	0.25
Trovafloxacin	0.06	0.06	8	4	0.06	0.06	0.06	0.125	0.125	0.06	0.06	0.125
Amoxicillin	0.016	0.016	0.008	0.008	0.03	0.125	0.125	0.06	1	1	2	2
Cefuroxime	0.5	0.25	0.016	0.016	0.5	0.5	0.5	0.25	2	0.5	4	2
Azithromycin	0.008	0.06	>64	0.125	>64	0.03	0.125	0.125	>64	>64	0.125	>64
Clarithromycin	0.008	0.03	>64	0.03	32	0.008	0.016	0.03	>64	>64	0.03	>64

[a]S = penicillin susceptible; I = penicillin intermediate; R = penicillin resistant.
[b]Quinolone-resistant.

TABLE 18. Time-kill results of 12 pneumococcal strains

Drug	3 h			6 h			12 h			24 h		
	-1[a]	-2[a]	-3[a]	-1	-2	-3	-1	-2	-3	-1	-2	-3
Gemifloxacin												
8 x MIC	10[b]	2	0	12	8	2	12	12	9	12	12	12
4 x MIC	9	1	0	12	8	0	12	12	8	12	12	12
2 x MIC	6	0	0	12	7	0	12	11	8	12	12	12
MIC	4	1	0	11	2	0	12	8	3	12	10	8
0.5 x MIC	1	0	0	4	0	0	3	0	0	2	2	0
0.25 x MIC	0	0	0	0	0	0	0	0	0	0	0	0
Ciprofloxacin												
8 x MIC	10	8	2	12	11	6	12	12	10	12	12	12
4 x MIC	9	6	1	12	10	5	12	12	10	12	12	12
2 x MIC	9	4	0	12	8	2	12	12	6	12	12	11
MIC	4	0	0	8	3	0	10	9	3	11	10	6
0.5 x MIC	0	0	0	1	1	0	2	1	0	2	1	0
0.25 x MIC	0	0	0	0	0	0	0	0	0	0	0	0
Levofloxacin												
8 x MIC	11	3	0	12	9	4	12	12	10	12	12	12
4 x MIC	10	4	0	12	9	1	12	12	8	12	12	12
2 x MIC	10	2	0	12	9	1	12	12	9	12	12	12
MIC	9	1	0	12	6	0	12	11	7	12	12	12
0.5 x MIC	4	1	0	8	1	0	7	3	0	8	7	5
0.25 x MIC	0	0	0	0	0	0	0	0	0	0	0	0

TABLE 18. (Continued)

Sparfloxacin												
8 x MIC	10	2	0	12	9	4	12	12	9	12	12	12
4 x MIC	9	1	0	12	8	0	12	11	8	12	12	12
2 x MIC	8	1	0	12	4	0	12	10	5	12	12	12
MIC	4	0	0	8	2	0	11	9	4	11	11	10
0.5 x MIC	1	0	0	5	1	0	4	0	0	6	4	1
0.25 x MIC	0	0	0	0	0	0	0	0	0	1	1	1
Grepafloxacin												
8 x MIC	8	2	1	12	5	2	12	11	7	12	12	12
4 x MIC	6	0	0	12	4	0	12	10	5	12	12	12
2 x MIC	3	0	0	9	1	0	10	8	1	11	10	9
MIC	1	0	0	4	1	0	7	3	0	8	5	3
0.5 x MIC	0	0	0	0	0	0	1	0	0	1	1	0
0.25 x MIC	0	0	0	0	0	0	0	0	0	0	0	0
Trovafloxacin												
8 x MIC	12	3	0	12	10	1	12	12	9	12	12	12
4 x MIC	9	2	0	12	9	1	12	10	8	12	12	12
2 x MIC	5	1	0	11	4	0	12	11	7	11	11	11
MIC	4	0	0	6	2	0	7	4	1	6	1	1
0.5 x MIC	0	0	0	0	0	0	0	0	0	0	0	0
0.25 x MIC	0	0	0	0	0	0	0	0	0	0	0	0
Amoxicillin												
8 x MIC	9	4	0	11	8	3	12	12	10	12	12	12
4 x MIC	7	2	0	12	7	0	12	12	9	12	12	12
2 x MIC	6	2	0	11	5	1	12	11	8	12	12	12
MIC	4	0	0	6	1	0	7	6	2	10	9	7
0.5 x MIC	0	0	0	0	0	0	1	1	0	3	2	1
0.25 x MIC	0	0	0	0	0	0	0	0	0	1	0	0

TABLE 18. (Continued)

Cefuroxime												
8 x MIC	9	5	0	12	12	4	12	12	12	12	12	12
4 x MIC	9	3	0	12	11	1	12	12	11	12	12	12
2 x MIC	7	1	0	12	8	1	12	11	8	12	12	11
MIC	4	0	0	7	2	0	9	7	1	9	9	9
0.5 x MIC	3	0	0	1	0	0	0	0	0	1	1	0
0.25 x MIC	0	0	0	0	0	0	0	0	0	0	0	0
Azithromycin[c]												
8 x MIC	3	1	0	6	4	2	7	5	5	7	7	7
4 x MIC	4	1	0	6	3	2	6	5	4	7	7	7
2 x MIC	3	1	0	4	2	2	5	5	4	7	7	5
MIC	2	0	0	3	2	2	5	5	2	7	6	5
0.5 x MIC	1	0	0	1	1	1	1	1	1	1	1	1
0.25 x MIC	0	0	0	0	0	0	0	0	0	0	0	0
Clarithromycin[c]												
8 x MIC	4	2	0	7	2	2	5	5	5	7	7	7
4 x MIC	3	2	0	7	2	2	5	5	5	7	7	7
2 x MIC	3	2	0	5	2	2	5	5	4	7	7	7
MIC	3	1	0	5	0	1	5	5	2	7	7	5
0.5 x MIC	1	0	0	3	0	0	1	1	1	4	3	1
0.25 x MIC	0	0	0	0	0	0	0	0	0	0	0	0

[a]ÄLog10 cfu/ml lower than 0 h.
[b]No. strains tested.
[c]Only 7 strains with macrolide MICs ≤0.125 μg/ml were tested.

The invention provides a method for modulating metabolism of pneumococcal pathogenic bacteria. Skilled artisans can readily choose pneumococcal pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by pneumococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with pneumococcal pathogenic bacteria.

While a preferred object of the invention provides a method wherein said pneumococcal pathogenic bacteria is selected from the group consisting of: bacteria comprising a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB; bacteria comprising a mutation in ParC at S79-F or Y, D83-N, R95-C, or K137-N; bacteria comprising a mutation in gyrA at S83-A, C, F, or Y; E87-K; or S116-G; bacteria comprising a mutation in parE at D435-N or I460-V; bacteria comprising a mutation in gyrB at D435-N or E474-K; bacteria comprising at least four mutations in a QRDR or parC, gyrA, parE, and gyrB; bacteria comprising a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in ParC at S79-F or Y, D83-N, R95-C, or K137-N; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in gyrA at S83-A, C, F, or Y; E87-K; or S116-G; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in parE at D435-N or I460-V; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in gyrB at D435-N or E474-K; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise at least four mutations in a QRDR or parC, gyrA, parE, and gyrB; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a

combination thereof, that comprise a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB; *Streptococcus pneumoniae* bacteria comprising a mutation in ParC at S79-F or Y, D83-N, R95-C, or K137-N; *Streptococcus pneumoniae* bacteria comprising a mutation in gyrA at S83-A, C, F, or Y; E87-K; or S116-G; *Streptococcus pneumoniae* bacteria comprising a mutation in parE at D435-N or I460-V; *Streptococcus pneumoniae* bacteria comprising a mutation in gyrB at D435-N or E474-K; *Streptococcus pneumoniae* bacteria comprising at least four mutations in a QRDR or parC, gyrA, parE, and gyrB; and *Streptococcus pneumoniae* bacteria comprising a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB.

Other pneumococcal pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

(F) Methods of Using Fluoroquinolones Against Ciprofloxacin-Resistant and Ciprofloxacin-Sensitive Bacteria

The present invention provides, among other things, methods for using a composition comprising a gemifloxacin compound against a ciprofloxacin resistant strain of *S. pneumoniae, S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae,* a trovafloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a fluoroquinolone resistant strain of *S. pneumoniae,* a fluoroquinolone resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, and a fluoroquinolone resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various strains of *S. pneumoniae*. An objective of these analyses was to determine the postantibiotic effect (herein "PAE") of GFX in CFX and TFX susceptible and resistant *S. pneumoniae*.

CFX resistant, clinical isolates of *S. pneumoniae* were collected from across Canada. MICs were determined using a microbroth dilution technique described by the NCCLS. Topisomeriase *IV (parC)* and DNA gyrase (*gyrA*) mutations were confirmed by sequencing the QRDR region of each gene. Two fluoroquinolone susceptible and 8 resistant isolates (3 CFX resistant, 5 CFX, TFX resistant) were selected for study. The PAE was determined by exposing logarithmic phase organisms at 4x or 10x MIC for 2 hours. Antibiotics were removed using dilution into sterile media and the PAE assessed using a viable colony counting technique. The MICs of CFX ranged from 0.5 μg/ml to 64 μg/ml and the MICs of TFX ranged from 0.06 μg/ml to 8.0 μg/ml. The MICs of GFX ranged from ≤ 0.03 μg/ml to 0.5 μg/ml. The mean PAE of CFX in susceptible *S. pneumoniae* was 1.6 hrs at 4x MIC and 2.5 hrs at 10x MIC. In TFX susceptible isolates, the mean PAE at 4x MIC was 2.1 hrs and 3.2 hrs at 10x MIC. The mean PAE of GFX was 2.7 hrs at 4x MIC and 3.8 hrs at 10x MIC. There was no significant difference in the duration of the GFX PAE between CFX or TFX susceptible and resistant strains ($p<0.05$). In conclusion, GFX remains highly active against CFX and TFX susceptible and resistant *S. pneumoniae* and produces a prolonged PAE in organisms displaying diminished susceptibility to other fluoroquinolones.

In another analysis, clinical isolates of *S.pneumoniae* were collected across Canada and isolates having an MIC to CIP of ≥ 2μg/ml were selected for further study. MICs to penicillin (herein "PEN"), CIP, levofloxacin (herein "LEV"), TFX, moxifloxacin (herein "MOX"), grepafloxacin (herein "GRE"), gatifloxacin (herein "GAT"), sparfloxacin (herein "SPA"), and gemifloxacin (herein "GFX") were determined using a microbroth dilution technique described by the NCCLS. Topoisomerase IV (parC) and DNA gyrase (gyrA) mutations were confirmed by sequencing the QRDR region of each gene. Serotyping and PFGE were performed on all isolates. In total, 80 isolates were identified with CIP MICs ≥ 2 μg/ml. Of these, 33 had both gyrA and parC mutations, 29 had parC mutations alone and 2 had gyrA mutations. With the exception of 7 isolates, all organisms having a CIP MIC ≥ 8 μg/ml, had both a parC and gyrA mutation. $MIC_{50/90s}$ are listed in Table 19. Breakpoints have not been established for all fluoroquinolones, thus percentage resistance was not calculated. With the exception of one cluster, serotyping and PFGE suggest that resistance is *de novo* and not due to clonal dissemination.

These results demonstrate that GFX, followed by MOX retain the greatest activity against *S.pneumoniae* with reduced susceptibility to CIP. The increasing use of fluoroquine to treat *S.pneumoniae* mandates continued surveillance to monitor changes in fluoroquine resistance patterns.

TABLE 19

μg/ml	CIP	LEV	SPA	TFX	MOX	GAT	GRE	GFX
MIC_{50}	8	2	0.5	0.5	0.25	0.5	0.5	0.06
MIC_{90}	32	16	16	4	2	4	8	0.25
range	2-64	1-32	.25-32	.06-8	.12-4	.25-8	.12-8	.03-.5

The invention provides a method for modulating metabolism of fluoroquinolone resistant pathogenic bacteria. Skilled artisans can readily choose fluoroquinolone resistant pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by fluoroquinolone resistant pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with fluoroquinolone resistant pathogenic bacteria.

While a preferred object of the invention provides a method wherein said fluoroquinolone resistant pathogenic bacteria is selected from the group consisting of: a ciprofloxacin resistant strain of *S. pneumoniae, S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae,* a trovafloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a fluoroquinolone resistant strain of *S. pneumoniae,* a fluoroquinolone resistant strain of *S. pneumoniae* having

a topisomerase *IV* (*parC*) mutation in the QRDR region, and a fluoroquinolone resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region. Other fluoroquinolone resistant pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

(G) Methods of Using Fluoroquinolones Against Bacteria Having Topoisomeras IV or Gyrase Mutations

The present invention provides, among other things, methods for using a composition comprising a gemifloxacin compound against a ciprofloxacin resistant strain of *S. pneumoniae, S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae,* a trovafloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a fluoroquinolone resistant strain of *S. pneumoniae,* a fluoroquinolone resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, and a fluoroquinolone resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various strains of *S. pneumoniae*. An objective of these analyses was to determine the postantibiotic effect (herein "PAE") of GFX in CFX and TFX susceptible and resistant *S. pneumoniae*.

CFX resistant, clinical isolates of *S. pneumoniae* were collected from across Canada. MICs were determined using a microbroth dilution technique described by the NCCLS. Topisomeriase *IV* (*parC*) and DNA gyrase (*gyrA*) mutations were confirmed by sequencing the QRDR region of each gene. Two fluoroquinolone susceptible and 8 resistant isolates (3 CFX resistant, 5 CFX, TFX resistant) were selected for study. The PAE was determined by exposing logarithmic phase organisms at 4x or 10x MIC for 2 hours. Antibiotics were removed using dilution into sterile media and the PAE assessed using a viable colony counting technique. The MICs of CFX ranged from 0.5 µg/ml to 64 µg/ml and the MICs of TFX ranged from 0.06 µg/ml to 8.0 µg/ml. The MICs of GFX ranged from

≤ 0.03 μg/ml to 0.5 μg/ml. The mean PAE of CFX in susceptible *S. pneumoniae* was 1.6 hrs at 4x MIC and 2.5 hrs at 10x MIC. In TFX susceptible isolates, the mean PAE at 4x MIC was 2.1 hrs and 3.2 hrs at 10x MIC. The mean PAE of GFX was 2.7 hrs at 4x MIC and 3.8 hrs at 10x MIC. There was no significant difference in the duration of the GFX PAE between CFX or TFX susceptible and resistant strains ($p<0.05$). In conclusion, GFX remains highly active against CFX and TFX susceptible and resistant *S. pneumoniae* and produces a prolonged PAE in organisms displaying diminished susceptibility to other fluoroquinolones.

In another analysis, clinical isolates of *S.pneumoniae* were collected across Canada and isolates having an MIC to CIP of ≥ 2μg/ml were selected for further study. MICs to penicillin (herein "PEN"), CIP, levofloxacin (herein "LEV"), TFX, moxifloxacin (herein "MOX"), grepafloxacin (herein "GRE"), gatifloxacin (herein "GAT"), sparfloxacin (herein "SPA"), and gemifloxacin (herein "GFX") were determined using a microbroth dilution technique described by the NCCLS. Topoisomerase IV (parC) and DNA gyrase (gyrA) mutations were confirmed by sequencing the QRDR region of each gene. Serotyping and PFGE were performed on all isolates. In total, 80 isolates were identified with CIP MICs ≥ 2 μg/ml. Of these, 33 had both gyrA and parC mutations, 29 had parC mutations alone and 2 had gyrA mutations. With the exception of 7 isolates, all organisms having a CIP MIC ≥ 8 μg/ml, had both a parC and gyrA mutation. $MIC_{50/90s}$ are listed in Table 20. Breakpoints have not been established for all fluoroquines, thus percentage resistance was not calculated. With the exception of one cluster, serotyping and PFGE suggest that resistance is *de novo* and not due to clonal disemmination.

These results demonstrate that GFX, followed by MOX retain the greatest activity against *S.pneumoniae* with reduced susceptibility to CIP. The increasing use of fluoroquine to treat *S.pneumoniae* mandates continued surveillance to monitor changes in fluoroquine resistance patterns.

TABLE 20

μg/ml	CIP	LEV	SPA	TFX	MOX	GAT	GRE	GFX
MIC_{50}	8	2	0.5	0.5	0.25	0.5	0.5	0.06
MIC_{90}	32	16	16	4	2	4	8	0.25
range	2-64	1-32	.25-32	.06-8	.12-4	.25-8	.12-8	.03-.5

The invention provides a method for modulating metabolism of fluoroquinolone resistant pathogenic bacteria. Skilled artisans can readily choose fluoroquinolone resistant pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by fluoroquinolone resistant pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with fluoroquinolone resistant pathogenic bacteria.

While a preferred object of the invention provides a method wherein said fluoroquinolone resistant pathogenic bacteria is selected from the group consisting of: a ciprofloxacin resistant strain of *S. pneumoniae, S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae,* a trovafloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a fluoroquinolone resistant strain of *S. pneumoniae,* a fluoroquinolone resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, and a fluoroquinolone resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region. Other fluoroquinolone resistant pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

(H) Methods of Using Fluoroquinolones Against Bacterial Infections Caused by Bacteria with QRDR Mutations

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against a quinolone-reisistant pneumococcal strain, particularly a strain comprising a mutation in the quinolone resistance-determining region (QRDR) of *parC* and/or *gyrA*; a pneumococcal strain comprising a

mutation in ParC said mutation comprising S79→F and/or Y, D83→G and/or N, N91→D, R95→C, and/or K137→N; a pneumococcal strain comprising a mutation in GyrA said mutation comprising S81→A, C, F, and/or Y; E85→K; and/or S114→G; a pneumococcal strain comprising a mutation in ParE said mutation comprising D435→N and/or I460→V; a pneumococcal strain comprising a mutation in GyrB said mutation comprising D435→N and/or E474→K; a pneumococcal strain comprising a mutation in comprising three or four mutations in a QRDRs of *parC*, *gyrA*, *parE*, and/or *gyrB*; a pneumococcal strain comprising a mutation in comprising three or four mutations in a QRDRs of *parC*, *gyrA*, *parE*, and/or *gyrB*, any of which are resistant to ciprofloxacin, levofloxacin, or sparfloxacin; and a pneumococcal strain comprising a mutation in comprising three or four mutations in a QRDRs of *parC*, *gyrA*, *parE*, and/or *gyrB*, any of which also comprising an efflux mechanism of quinolone resistance.

The present invention is based, in part, on experiments wherein *in vitro* activity of gemifloxacin was compared with that of ciprofloxacin, levofloxacin, sparfloxacin, grepafloxacin and trovafloxacin against 28 pneumococci with ciprofloxacin MICs ≥8 μg/ml. Gemifloxacin MICs (μg/ml) ranged between 0.03–1.0 ($MIC_{50/90}$ 0.25/0.5), compared with ciprofloxacin 8–>32 ($MIC_{50/90}$ 16/>32), levofloxacin 4–>32 ($MIC_{50/90}$ 16/>32), sparfloxacin 0.25–>32 ($MIC_{50/90}$ 8/16), grepafloxacin 0.5–16 ($MIC_{50/90}$ 4/8) and trovafloxacin 0.25– 8 ($MIC_{50/90}$ 1.0/4.0). DNA sequence analysis showed that all but one strain had a mutation in the quinolone resistance-determining region (QRDR) of *parC* and/or *gyrA*. Mutations in ParC were at S79→F or Y, D83→G or N, N91→D, R95→C, or K137→N. Mutations in GyrA were at S81→A, C, F, or Y; E85→K; or S114→G. Twenty-one strains had a mutation in ParE at D435→N or I460→V. Only two strains had a mutation in GyrB at D435→N or E474→K. Twenty-one strains had a total of three or four mutations in the QRDRs of *parC*, *gyrA*, *parE*, and *gyrB*. Of these 21 strains, all were resistant to ciprofloxacin (MIC ≥8 μg/ml), levofloxacin (MIC ≥4 μg/ml), and sparfloxacin (MIC ≥1 μg/ml); 20 were resistant to grepafloxacin (MIC ≥1 μg/ml) and 11 were resistant to trovafloxacin (MIC ≥2μg/ml), yet gemifloxacin MICs were ≤0.5 μg/ml in 19 of the strains. In the presence of reserpine, 23 strains had lower ciprofloxacin MICs (2–16x), 13 strains had lower gemifloxacin MICs (2–4x), 7 strains had lower levofloxacin MICs (2–4x); 3 strains had lower grepafloxacin MICs (2x) and one strain had lower sparfloxacin MICs (2x), indicating that an efflux mechanism contributed to the raised MICs in some

cases. Results show that, irrespective of the mechanism of quinolone resistance, gemifloxacin showed the greatest *in vitro* activity against all pneumococcal strains tested. Against 28 strains with ciprofloxacin MICs ≥8 μg/ml, gemifloxacin had the lowest MICs (0.03–1.0 μg/ml, MIC_{90} 0.5 μg/ml), compared with MICs ranging between 0.25 to >32.0 μg/ml (MIC_{90}s 4.0–>32.0 μg/ml) for the other quinolones, with trovafloxacin, grepafloxacin, sparfloxacin and levofloxacin, in ascending order, giving the next lowest MICs (Table 21). Mechanisms of quinolone resistance are presented in Tables 22 and 23. As can be seen, quinolone resistance was associated with mutations in the quinolone resistance-determining region (QRDR) of *parC*, *gyrA*, *parE* and/or *gyrB*. Mutations in ParC were at S79-F or Y, D83-N, R95-C, or K137-N. Mutations in *gyrA* were at S83-A, C, F, or Y; E87-K; or S116-G. Twenty-one strains had a mutation in *parE* at D435-N or I460-V. Only two strains had a mutation in *gyrB* at D435-N or E474-K. Twenty-one strains had a total of three or four mutations in the QRDRs of *parC*, *gyrA*, *parE* and *gyrB* (Table 22). Amongst these 21 strains all were resistant to ciprofloxacin (MICs ≥8 μg/ml), levofloxacin (MICs ≥4 μg/ml), and sparfloxacin (MICs ≥1 μg/ml), 20 were resistant to grepafloxacin (MICs ≥1 μg/ml) and 11 were resistant to trovafloxacin (MICs ≥2 μg/ml), yet gemifloxacin MICs were ≤ 0.5 μg/ml in 19 of the strains (Table 22).

In the presence of reserpine 23 strains had lower ciprofloxacin MICs (2–16 x), 13 strains had lower gemifloxacin MICs (2–4x), 7 strains had lower levofloxacin MICs (2–4x); 3 strains had lower grepafloxacin MICs (2x); and one strain had lower sparfloxacin MICs (2x), indicating that an efflux mechanism contributed to the raised MICs in some cases (Table 23). Previous studies have shown gemifloxacin to be 32 to 64 fold more active than ciprofloxacin, ofloxacin, sparfloxacin and trovafloxacin against methicillin-susceptible and -resistant *Staphylococcus aureus*, methicillin-resistant *Staphylococcus epidermidis* and *S. pneumoniae*. Gemifloxacin was also highly active against most members of the family *Enterobacteriaceae*, with activity which was more potent than those of sparfloxacin and ofloxacin and comparable to that of ciprofloxacin. Gemifloxacin was the most active agent against Gram positive species resistant to other quinolones and glycopeptides. Gemifloxacin has variable activity against anaerobes, and is very active against the Gram positive group (Cormican, et al., *Antimicrobiol. Agents Chemother.* 41:204-211, 1997; Hohl, et al., *Clin. Microbiol. Infect.* 4:280-284, 1998; Oh, et al., *Antimicrob. Agents Chemother.* 40:1564-1568, 1996).

In our study, gemifloxacin gave significantly lower MICs against highly quinolone-resistant pneumococci, irrespective of quinolone resistance mechanism. This was the case in double mutants with mutations in both *parC* and *gyrA*, strains which have previously been

shown to be highly resistant to other quinolones, as well as for strains with an efflux mechanism (Pan, et al., *Antimicrob. Agents Chemother.* 40:2321-2326, 1996 and Brenwald, et al., *Antimicrob. Agents Chemother.* 42:2032-2035, 1998).

In summary, gemifloxacin was the most potent quinolone tested against quinolone resistant pneumococci. The incidence of quinolone-resistant pneumococci is currently very low. However, this situation may change with the introduction of broad-spectrum quinolones into clinical practice, and in particular in the pediatric population, leading to selection of quinolone-resistant strains (Davies, et al., *Antimicrob. Agents Chemother.* 43:1177-1182, 1999). Results indicate that selective introduction of quinolones such as gemifloxacin into the pediatric environment is predicated upon toxicologic studies. Additionally, if the incidence of quinolone- resistant pneumococci increases, gemifloxacin will be a well-placed therapeutic option. Gemifloxacin is a promising new antipneumococcal agent, irrespective of the strain's susceptibility to quinolones and other agents.

Table 21. Quinolone Agar Dilution MICs (µg/ml) of 28 Ciprofloxacin-Resistant Strains (MICs ≥8.0 µg/ml)

Quinolone	MIC range	MIC_{50}	MIC_{90}
Gemifloxacin	0.3–1.0	0.25	0.5
Ciprofloxacin	8.0–>32.0	16.0	>32.0
Levofloxacin	4.0–>32.0	16.0	>32.0
Sparfloxacin	0.25–>32.0	8.0	16.0
Grepafloxacin	0.5–16.0	4.0	8.0
Trovafloxacin	0.25–8.0	1.0	4.0

Table 22. Correlation of Quinolone MIC (μg/ml) and Mutation in Quinolone-Resistant Strains

Strain	MIC (μg/ml)						Mutation			
	Gemifloxacin	Ciprofloxacin	Levofloxacin	Sparfloxacin	Grepafloxacin	Trovafloxacin	ParC	ParE	GyrA	GyrB
1	0.03	16	8	4	4	2	S79-F	I460-V	S81-F	None
2	0.06	8	4	0.5	0.5	0.25	S79-Y	I460-V	None	None
3	0.06	8	4	1	0.5	0.25	D83-N	I460-V	S81-F	None
4	0.06	8	4	1	1	0.25	S79-F	I460-V	S81-F	None
5	0.125	8	8	1	1	0.25	R95-C	D435-N	S81-F	None
6	0.125	8	8	8	2	2	S79-Y	I460-V	E85-K	None
7	0.125	8	8	1	1	0.5	None	I460-V	None	None
8	0.125	8	8	1	1	0.5	S79-Y	None	None	None
9	0.125	8	8	2	1	1	S79-Y	None	None	None
10	0.125	8	8	4	2	1	S79-F	I460-V	S81-C	None
11	0.125	8	8	4	4	1	S79-F	I460-V	S81-F	None
12	0.125	>32	16	1	4	1	S79-F	I460-V	None	D435-N
13	0.25	16	8	8	2	1	S79-F	I460-V	None	E474-K

Table 22. (Continued)

14	0.25	16	16	8	4	1	S79-F	I460-V	S81-F	None
15	0.25	16	16	8	4	1	S79-F	I460-V	S81-F	None
16	0.25	16	16	8	4	2	S79-F	I460-V	S81-F	None
17	0.25	16	16	8	4	2	D83-N	None	S81-F	None
18	0.25	16	16	8	4	2	S79-F	I460-V	S81-F	None
19	0.25	16	16	8	4	2	S79-F	I460-V	S81-F	None
20	0.25	32	16	8	4	2	S79-F	I460-V	E85-K	None
21	0.25	32	16	8	4	1	S79-F	I460-V	S81-F	None
22	0.25	32	16	8	4	2	S79-F	I460-V	S81-Y	None
23	0.25	32	16	8	8	2	S79-Y	None	S81-A	None
24	0.5	32	32	16	8	4	S79-F	I460-V	S81-F	None
25	0.5	32	32	16	8	4	D83-G N91-D	None	S81-F	None
26	0.5	>32	>32	>32	8	4	S79-F	None	S81-Y	None
27	1	>32	>32	>32	16	8	S79-Y	I460-V	S81-F	None
28	1	>32	>32	>32	16	8	D83-G N91-D	None	S81-F S114-G	None

Table 23. Efflux Mechanisms in Quinolone-Resistant Pneumococci

Strain	Gemifloxacin	Ciprofloxacin	Levofloxacin	Sparfloxacin	Grepafloxacin	Trovafloxacin
1	2 X[a]	2 X	-	-	-	-
2	-	8 x	-	-	-	-
3	-	-	-	-	-	-
4	-	-	-	-	-	-
5	2 X	4 X	-	2 X	2 X	-
6	-	2 X	-	-	-	-
7	2 X	4 X	2 X	-	-	-
8	2 X	2 X	-	-	-	-
9	4 X	8 X	-	-	-	-
10	-	2 X	-	-	-	-
11	2 X	16 X	4 X	-	-	-
12	2 X	4 X	2 X	-	2 X	-
13	-	2 X	-	-	-	-
14	-	-	-	-	-	-
15	-	-	-	-	-	-
16	2 X	4 X	-	-	-	-
17	2 X	4 X	-	-	-	-
18	-	2 X	-	-	-	-
19	-	2 X	-	-	-	-
20	-	2 X	-	-	-	-
21	-	2 X	-	-	-	-
22	-	2 X	-	-	-	-
23	2 X	8 X	2 X	-	-	-

Table 23. (Continued)

24	-	2 x	-	-	-	-
25	2 x	4 x	2 x	-	-	-
26	2 x	4 x	2 x	-	-	-
27	-	-	-	-	-	-
28	4 x	8 x	4 x	-	2 X	-

[a]Number of dilutions decrease after incubation with reserpine (see Materials and Methods). National Committee for Clinical Laboratory Standards. 1997. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically -- third edition; approved standard. NCCLS publication no. M7-A4. National Committee for Clinical Laboratory Standards, Villanova, PA.

The invention provides a method for modulating metabolism of quinolone-resistant pneumococcal pathogenic bacteria. Skilled artisans can readily choose quinolone-resistant pneumococcal pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by quinolone-resistant pneumococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with quinolone-resistant pneumococcal pathogenic bacteria.

While a preferred object of the invention provides a method wherein said quinolone-resistant pneumococcal pathogenic bacteria is selected from the group consisting of: a pneumococcal strain comprising a mutation in the quinolone resistance-determining region (QRDR) of *parC* and/or *gyrA*; a pneumococcal strain comprising a mutation in ParC said mutation comprising S79→F and/or Y, D83→G and/or N, N91→D, R95→C, and/or K137→N; a pneumococcal strain comprising a mutation in GyrA said mutation comprising S81→A, C, F, and/or Y; E85→K; and/or S114→G; a pneumococcal strain comprising a mutation in ParE said mutation comprising D435→N and/or I460→V; a pneumococcal strain comprising a mutation in GyrB said mutation comprising D435→N and/or E474→K; a pneumococcal strain comprising a mutation in comprising three or four mutations in a QRDRs of *parC*, *gyrA*, *parE*, and/or *gyrB*; a pneumococcal strain comprising a mutation in comprising three or four mutations in a QRDRs of *parC*, *gyrA*, *parE*, and/or *gyrB*, any of which are resistant to ciprofloxacin, levofloxacin, or sparfloxacin; and a pneumococcal strain comprising a mutation in comprising three or four mutations in a QRDRs of *parC*, *gyrA*, *parE*, and/or *gyrB*, any of which also comprising an efflux mechanism of quinolone resistance.

Other quinolone-resistant pneumococcal pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

(I) Methods of Using Fluoroquinolones Against Mutant *Haemophilus* Bacteria

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against bacteria comprising a mutation set forth in Table 25 or 26; a *Haemophilus influenzae* strain set forth in Table 25 or 26; bacteria of the genus *Haemophilus* comprising a mutation set forth in Table 25 or 26; or bacteria of the species *Haemophilus influenzae* comprising a mutation set forth in Table 25 or 26.

Nine quinolone-resistant *H. influenzae* strains yielded MIC_{50}s of 0.25 μg/ml for gemifloxacin (highest MIC 1.0 μg/ml) compared to 1.0 μg/ml (highest MIC 4.0–8.0 μg/ml) for the other quinolones tested (Table 24). Mechanisms of quinolone resistance in the *H. influenzae* strains are presented in Table 25. All nine strains had mutations at Ser 84 in GyrA with Ser 84 to Leu, Phe, or Tyr observed. Additional mutations in GyrA at Asp 88 to Asn or Tyr, and Ala 117 to Glu were also observed in some strains. Most strains also had at least one mutation in ParC (at Asp 83, Ser 84, Glu 88, Ser 133, or Asn 138) and ParE (at Gly 405, Asp 420, Ser 458, or Ser 474). Strain 4 had an in-frame insertion in *parE* that led to an insertion of a Ser residue in between Ser 458 and Thr 459. Only one strain had a mutation in GyrB (at Gln 468). The most resistant strain (strain 9) had double mutations in GyrA, ParC and ParE.

Previous studies have shown gemifloxacin to be 32–64 fold more active than ciprofloxacin, ofloxacin, sparfloxacin and trovafloxacin against methicillin-susceptible and -resistant *S. aureus*, methicillin-resistant *Staphylococcus epidermidis* and *S. pneumoniae*. Gemifloxacin was also highly active against most members of the family *Enterobacteriaceae*, with activity more potent than those of sparfloxacin and ofloxacin and comparable to that of ciprofloxacin. Gemifloxacin was the most active agent against Gram positive species resistant to other quinolones and glycopeptides. Gemifloxacin has variable activity against anaerobes and is very active against the Gram positive group (Cormican, et al., *Antimicrob. Agents Chemother.* 41:204-211, 1997; Hohl, et al., *Clin. Microbiol. Infect.* 4:280-284, 1998; and Oh, et al., *Antimicrob. Agents Chemother.* 40:1564-1568, 1996. In the studies set forth herein, only gemifloxacin gave MICs ≤1.0 μg/ml against the rare strains of *H. influenzae* with raised quinolone MICs. Previous studies (Bootsma, et al., *J. Antimicrob. Chemother.* 39:292-293, 1997; Georgiou, et al., *Antimicrob. Agents Chemother.*, 40:1741-1744, 1996; and Vila, et al.,

Antimicrob. Agents Chemother., 43:161-162, 1999) have shown that the primary target of quinolones in *H. influenzae* is GyrA; low-level resistance is associated with a mutation in GyrA (Ser 84 or Asp 88) and high-level resistance with an additional mutation in ParC (Asp 83, Ser 84 or Glu 88). Sequencing results from this study were in agreement with the above previous reports, as all nine strains had at least one mutation in GyrA and the most resistant strains (ciprofloxacin MICs ≥1.0 μg/ml) had an additional mutation in ParC. Mutations were found in GyrA (Ala 117) and ParC (Ser 133, Asn 138) that have not been previously reported. Provided herein is a novel examination of mutations in GyrB and ParE in *H. influenzae*: most strains had mutations in ParE, but only one strain in GyrB. Of particular interest was insertion of a serine between serine 458 and threonine 459 of ParE in one strain. It, therefore, appears that ParE is more important in quinolone resistance in *H. influenzae* than GyrB.

Results of this study indicate excellent activity of gemifloxacin against quinolone-resistant *H. influenzae* (including those with multiple mutations) by MIC. Because of the wide spectrum of activity of gemifloxacin against other respiratory pathogens, such as pneumococci (including quinolone-resistant strains), *Legionella*, mycoplasmas and chlamydia, this compound represents an attractive alternative to other quinolone and non-quinolone agents for empiric treatment of community-acquired respiratory tract infections.

Table 24. Quinolone MICs (μg/ml) for 9 Quinolone-Resistant *Haemophilus influenzae* strains

Antimicrobial	Range	MIC_{50}
Gemifloxacin	0.03–1.0	0.25
Ciprofloxacin	0.25–8.0	1.0
Levofloxacin	0.25–4.0	1.0
Sparfloxacin	0.25–8.0	1.0
Grepafloxacin	0.25–4.0	1.0
Trovafloxacin	0.25–8.0	1.0

Table 25. Mechanisms of Resistance in Quinolone-Resistant *Haemophilus influenzae* strains

Strain	MIC (μg/ml)						Mutation			
	Gemi	Cipro	Levo	Spar	Grepa	Trova				
1	0.03	0.5	0.5	0.25	0.25	1	S133-A N138-S	None	S84-L	None
2	0.125	0.25	0.25	0.25	0.5	0.25	NONE	S458-L	S84-F	None
3	0.125	1	1	0.5	0.5	1	S84-I	None	S84-L	None
4	0.25	1	0.5	0.25	2	0.5	D83-N	S458-S-T459	S84-F D88-N	Q468-R
5	0.25	1	1	1	1	1	E88-K	G405-S	S84-Y	None
6	0.5	2	2	1	1	4	S84-R	D420-N	S84-L A117-E	None
7	0.5	2	2	1	1	4	S84-R	D420-N	S84-L A117-E	None
8	0.5	2	2	1	1	4	S84-R	D420-N	S84-L A117-E	None
9	1	8	4	8	4	8	S84-R N138-S	S458-A S474-N	S84-F D88-Y	None

TABLE 26

	MICs (μg/ml)						Mutations			
Strain	Gem	Cip	Lev	Spa	Gre	Tro	GyrA	ParC	GyrB	ParE
1	0.03	0.5	0.5	0.25	0.25	1	S84→L	S133→A N138→S	None	None
2	0.125	0.25	0.25	0.25	0.5	0.25	S84→F	None	None	S458→L
3	0.125	1	1	0.5	0.5	1	S84→L	S84→I	None	None
4	0.25	1	0.5	0.25	2	0.5	S84→F D88→N	D83→N	None	T459→S
5	0.25	1	1	1	1	1	S84→Y	E88→K	Q468→R	G405→S
6	0.5	2	2	1	1	4	S84→L A117→E	S84→R	None	D420→N
7	0.5	2	2	1	1	4	S84→L A117→E	S84→R	None	D420→N
8	0.5	2	2	1	1	4	S84→L A117→E	S84→R	None	D420→N
9	1	8	4	8	4	8	S84→F D88→Y	S84→R N138→S	None	S458→A S474→N

All strains had mutations at position 84 in *gyrA*, and the most R strain had double mutations in *gyrA*, *parC* and *parE*. Strains with mutations at position 84 in *parC* and *gyrA* plus mutations in *parE* were tro R. Gem had the lowest MICs against all strains irrespective of their mutation mechanism.

The invention provides a method for modulating metabolism of a rare pathogenic H. influenzae strain. Skilled artisans can readily choose a rare pathogenic H. influenzae strain or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by a rare pathogenic H. influenzae strain comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a

gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with a rare pathogenic H. influenzae strain.

While a preferred object of the invention provides a method wherein said rare pathogenic H. influenzae strain is selected from the group consisting of: bacteria comprising a mutation set forth in Table 25 or 26; a *Haemophilus influenzae* strain set forth in Table 25 or 26; bacteria of the genus *Haemophilus* comprising a mutation set forth in Table 25 or 26; and bacteria of the species *Haemophilus influenzae* comprising a mutation set forth in Table 25 or 26.

Other rare pathogenic H. influenzae strains may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

(J) Methods of Using Fluoroquinolones Against Ciprofloxacin-Resistant and Ciprofloxacin-Sensitive *S. pneumonia* Bacteria

The present invention provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against ciprofloxacin-susceptible pneumococci having an MIC ≤ 4 μg/ml of ciprofloxacin; ciprofloxacin-resistant pneumococci having an MIC ≥ 8 μg/ml of ciprofloxacin; ciprofloxacin-susceptible *Streptococcus pneumoniae* having an MIC ≤ 4 μg/ml of ciprofloxacin; and ciprofloxacin-resistant *Streptococcus pneumoniae* having an MIC ≥ 8 μg/ml of ciprofloxacin.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various baterial pathogens.

The invention was based, in part, on experiments using agar dilution, microdilution (both in air), E-test and disk diffusion (both in air and CO_2) were used to test activity of gemifloxacin (SB-265805) against 161 ciprofloxacin-susceptible (cipro-S) (MIC ≤ 4 μg/ml) and 39 ciprofloxacin-resistant (cipro-R) (MIC ≥ 8 μg/ml) pneumococci. By agar, gemifloxacin MIC_{50}s/MIC_{90}s (μg/ml) for cipro-S and -R strains were 0.03/0.03 and 0.25/0.5, respectively. Results of agar dilution MICs for ciprofloxacin *vs* gemifloxacin for all 200 strains showed a linear correlation. Comparing the three MIC methods for all strains, MICs (μg/ml) were practically identical: agar dilution, range 0.004–1, MIC_{50} 0.03, MIC_{90} 0.25; Microdilution, range 0.004–0.5, MIC_{50} 0.016, MIC_{90} 0.125; E-test (air), range 0.008–0.5, MIC_{50} 0.016, MIC_{90} 0.125; E-test (CO_2), range 0.008–0.5, MIC_{50} 0.016, MIC_{90} 0.25. With agar dilution as the standard, 187/200 strains (93.5%) gave essential agreement (± 1 $\log_2$ dilution) with

microdilution, and 196/200 (98%) with the E-test (both in air and CO_2). Incubation of E-tests in air and CO_2 gave identical results. With a 0.5 µg/ml breakpoint, no major or very major errors occurred. When 5 µg gemifloxacin disks were incubated in CO_2, all cipro-S strains yielded gemifloxacin diameters ≥26 mm. Gemifloxacin diameters in CO_2 for cipro-R strains varied between 18–31 but were mostly 21–26 mm. Using a gemifloxacin breakpoint of 0.5 µg/ml, diameters in CO_2 of ≥20 mm for S and ≤19 mm for R strains are proposed: all strains but one (with an agar dilution MIC 1 µg/ml) had gemifloxacin MICs of ≤0.5 µg/ml, and all but one had zone diameters in CO_2 >20 mm. Most diameters in air were 1–3 mm wider than in CO_2, but S and R results were identical. Results show that i) Gemifloxacin is very active against cipro-S and -R strains; ii) susceptibility to gemifloxacin can be reliably tested by agar and microdilution, E-test and disk diffusion; iii) CO_2 does not significantly affect gemifloxacin pneumococcal susceptibility results.

Results of MIC testing of gemifloxacin with the four methods used are presented in Table 27, and results (agar dilution MIC) broken down by ciprofloxacin susceptibility in Table 28. By agar dilution, ciprofloxacin MICs (µg/ml) for all strains ranged between 0.5–≥64, with an MIC_{50} of 2 and an MIC_{90} of 16. By contrast, gemifloxacin MICs, which were practically identical with all methods, ranged between 0.004–1.0 µg/ml, with MIC_{50}s between 0.016–0.03 µg/ml and MIC_{90}s between 0.125–0.25 µg/ml with agar dilution and microdilution in air, and E-test (both in air and CO_2). Incubation of E-tests in CO_2 did not significantly influence MICs. When strains with ciprofloxacin MICs ≤4.0 µg/ml) were separated from strains with ciprofloxacin MICs ≥8 µg/ml, gemifloxacin $MIC_{50/90}$ values (µg/ml) by agar dilution were 0.03/0.03 and 0.25/0.5, respectively. By contrast, ciprofloxacin $MIC_{50/90}$ values (µg/ml) for susceptible and resistant strains were 1/2 and 32/≥64, respectively (Table 28). Results of agar dilution MICs for ciprofloxacin *vs* gemifloxacin for all 200 strains tested showed a linear correlation (Figure 1).

Agreements of microdilution and E-test (air and CO_2) with agar dilution (used as the reference method) and E-tests in air versus CO_2 are presented in Table 29. As can be seen, 187/200 strains (93.5%) gave essential agreement (±1 $\log_2$ dilution) with microdilution, and 98.0% with the E-test (both in air and CO_2). With a preliminary breakpoint of 0.5 µg/ml, no major or very major discrepancies were found with microdilution in air or E-test in air or CO_2. E-tests incubated in air gave virtually identical results to those in CO_2 (Table 29).

With disks incubated in CO_2, all quinolone-susceptible strains yielded zone diameters ≥26 mm; values in air were ≥28 mm. Zone diameters for quinolone-resistant strains in CO_2

varied between 18 and 31 mm but were mostly 21–26 mm; zone diameters in air were a few mm wider, but were also mostly <31 mm. Correlation between microdilution in air, the method recommended by NCCLS, and disk diffusion (incubated in CO_2) is presented in Figure 2. Using a gemifloxacin breakpoint of 0.5 μg/ml, ≥20 mm for susceptible and ≤19 mm (resistant) are proposed, as all strains but one (with agar dilution) had MICs of ≤0.5 μg/ml, and all but one strain had zones >20 mm. With a breakpoint of 0.25 μg/ml, zone diameters (mm) of ≥23 (susceptible), 21–22 (intermediate) and ≤20 (resistant) are indictated. Zone diameters in air were usually 1–3 mm wider than those in CO_2.

MICs of gemifloxacin against pneumococci are similar to those described previously, including MICs (μg/ml) of 0.004–0.06 if ciprofloxacin MICs are ≤4 (Oh, et al., *Antimicrob. Agents Chemother.* 40:1564-1568, 1996; Cormican, et al., *Antimicrob. Agents Chemother.*, 41:204-211, 1997; Hohl, et al., *Clin.Microbiol. Infect.* 4:280-284, 1998; and Kelly, et al., *Program and Abstracts of the Thirty-Eighth Interscience Conference on Microbial Agents and Chemotherapy,* San Diego, CA, USA 1998. American Society for Microbiology: Washington, DC, 1998, page 254, Abstract F-87) and 0.03–1.0 if ciprofloxacin MICs are 8.0–64.0. Additionally, MICs did not differ significantly with agar and microdilution incubated in air, and E-tests incubated in air and CO_2. Other studies demonstrated the same findings with levofloxacin (Clark, et al., *J. Clin. Microbiol.* 36:3579-3584, 1998). Disk diffusion testing showed zone sizes slightly smaller in CO_2 than in air. Determination of breakpoints, both by disk diffusion and MIC, must await further studies, but ciprofloxacin-resistant strains with gemifloxacin MICs of 0.06–1.0 μg/ml by agar dilution gave zone diameters in CO_2 between 21 and 31 mm (with the exception of one strain with a zone diameter of 18 mm), and most quinolone-susceptible strains yielded zone diameters >25 mm.

Results of this study indicate an excellent correlation between agar dilution, microdilution and E-test methods, and all methods can confidently be recommended for pneumococcal susceptibility testing with gemifloxacin. Using a gemifloxacin breakpoint of 0.5 μg/ml, ≥20 mm for susceptible and ≤19 mm are proposed (see Results and Figure 2). With a breakpoint of 0.25 μg/ml, zone diameters (mm) of ≥23 (susceptible), 21–22 (intermediate) and ≤20 (resistant) are indicated.

Table 27. Gemifloxacin MICs (μg/ml) with the three methods tested against 200 strains

Method	MIC range	MIC_{50}	MIC_{90}
Agar dilution (air)	0.004–1.0	0.03	0.25
Microdilution (air)	0.004–0.5	0.016	0.125
E-test (air)	0.008–0.5	0.016	0.125
E-test (CO_2)	0.008–0.5	0.016	0.25

Table 28. Comparison of agar dilution MIC results for ciprofloxacin-susceptible (161) and -resistant (39) strains

Antimicrobial	Ciprofloxacin MIC ≤4.0 (μg/ml)			Ciprofloxacin MIC ≥8.0 (μg/ml)		
	Range	MIC_{50}	MIC_{90}	Range	MIC_{50}	MIC_{90}
Gemifloxacin	0.004–0.06	0.03	0.03	0.03–1	0.25	0.5
Ciprofloxacin	0.5–4	1	2	8–≥64	32	≥64

Table 29. Results of gemifloxacin pneumococcal susceptibility testing by four methods using agar dilution as the reference method

Method		Number of strains with $\log_2$ ratios of reference to test MICs of method A *vs* method B							Number ± 1 $\log_2$ dilution
Method A	Method B	$\geq +3$	+2	+1	0	-1	-2	-3	
Agar dilution	Microdilution	0	0	5	83	99	13	0	187
Agar dilution	E-test (air)	0	0	7	87	102	4	0	196
Agar dilution	E-test (CO_2)	0	0	12	95	89	4	0	196
E-test (air)	E-test (CO_2)	0	0	27	164	9	0	0	200

The invention provides a method for modulating metabolism of ciprofloxacin-resistant and/or ciprofloxicin-sensitive bacteria. Skilled artisans can readily choose ciprofloxacin-resistant and/or ciprofloxicin-sensitive bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by ciprofloxacin-resistant and/or ciprofloxicin-sensitive bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with ciprofloxacin-resistant and/or ciprofloxicin-sensitive bacteria.

While a preferred object of the invention provides a method wherein said ciprofloxacin-resistant and/or ciprofloxicin-sensitive bacteria is selected from the group consisting of: ciprofloxacin-susceptible pneumococci having an MIC ≤4 μg/ml of ciprofloxacin; ciprofloxacin-resistant pneumococci having an MIC ≥8 μg/ml of ciprofloxacin; ciprofloxacin-susceptible *Streptococcus pneumoniae* having an MIC ≤4 μg/ml of ciprofloxacin; and ciprofloxacin-resistant *Streptococcus pneumoniae* having an MIC ≥8 μg/ml of ciprofloxacin. Other ciprofloxacin-resistant and/or ciprofloxicin-sensitive bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The invention also provides the use of a gemifloxacin compound, or a pharmaceutically acceptable derivative thereof, in the manufacture of a medicament for the treatment of infections caused by any of the bacteria mentioned hereinabove.

Preferred embodiments of the invention include, among other things, methods wherein said composition comprises gemifloxacin, or a pharmaceutically acceptable derivative thereof. Particularly preferred composisions comprise gemifloxacin mesylate, and hydrates thereof, particularly gemifloxacin sesquihydates thereof.

(K) Methods of Using Fluoroquinolones Against Ciprofloxacin-Resistant and Trovafloxacin-Reistant Bacteria

The present invention also provides, among other things, methods for using a composition comprising a quinolone, particularly a gemifloxacin compound against a bacteria with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a respiratory tract pathogenic bacteria with elevated MICs to or otherwise resistant to ciprofloxacin or

trovafloxacin, a member of the genus *Streptococcus* with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a *Streptococcus pneumoniae* strain with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a penicillin-resistant member of the genus *Streptococcus* with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, or a penicillin-resistant *Streptococcus pneumoniae* strain with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin.

This invention was based, in part, on analyses evaluating the comparative activity of gemifloxacin against various ciprofloxacin-resistant, trovafloxacin-resistant and other pathogens. An objective of these analyses was to determine the *in vitro* activity of a gemifloxacin compound in comparison with trovafloxacin against *Streptococcus pneumoniae* strains with elevated MICs to ciprofloxacin.

A nationwide surveillance study involving 14 centres in Spain collected a total of 1113 *S. pneumoniae* isolates from patients with community-acquired respiratory tract infections from May 1996 to April 1997. Of these, 39 isolates (3.5%) exhibited high (elevated) ciprofloxacin MICs (≥4 mg/L). The comparative *in vitro* activity of two new quinolones, gemifloxacin and trovafloxacin, was determined in triplicate against the ciprofloxacin-resistant strains using standard National Committee for Clinical Laboratory Standards (NCCLS) broth microdilution methods. The susceptibilities (MIC data; modal values) of these strains are summarised below (Table 30):

Table 30

Antimicrobial	MIC (mg/L)			% strains with	
	MIC_{50}	MIC_{90}	Range	MIC ≥2	MIC ≥4
Ciprofloxacin	4	8	4–≥16	100	100
Trovafloxacin	0.25	2	0.03–4	12.8	2.5
Gemifloxacin	0.03	0.06	0.03–0.25	0	0

Of the 39 strains, 59%, 13% and 28% were penicillin-susceptible, intermediate and penicillin-resistant, respectively. Gemifloxacin exhibited greater activity, with an MIC_{90} 33-fold lower than trovafloxacin and 133-fold lower than ciprofloxacin. No correlation was observed between quinolone and penicillin resistance in the *S. pneumoniae* isolates.

The high incidence of penicillin-resistant *S. pneumoniae* in Spain (36%) together with emerging resistance to 'older' quinolones indicates that the new quinolones should be included in pneumococcal susceptibility surveillance studies.

Penicillin resistance among pneumococci has increased in Spain from 44% in a national survey carried out in 1979–89[1] to 60% in 1996–97.[2] This has been due to the rise in prevalence of high-level resistance (MIC ≥2 mg/L) (15.3% in 1979–89[1] versus 36.5% in 1996–97[2]). The incidence of erythromycin resistance has also increased, from 10% in 1979–89[1] to 33.7% in 1996–97:[2] as the proportion of highly resistant (MIC ≥8 mg/L) strains has grown from 9.4% in 1990[3] to 25.8% in 1996–97.[2] In Spain, 50% of the global antibiotic consumption comprises penicillins and 17% is made up of cephalosporins. New quinolones have therefore became a therapeutic alternative in an environment of increasing penicillin/macrolide resistance, particularly when highly resistant strains are present and when macrolide resistance is associated with penicillin resistance.[2]

The aim of the analysis provided herein was to determine the incidence of ciprofloxacin-resistant (MIC ≥4 mg/L) strains of *Streptococcus pneumoniae* and to study the *in vitro* susceptibility of those strains to trovafloxacin and gemifloxacin in a nationwide surveillance study. This analysis served, in part, as the basis for the present invention.

The incidence of ciprofloxacin resistance (MIC ≥4 mg/L) was 3.5% (39 of 1113 strains). Comparisons of the *in vitro* susceptibility of gemifloxacin versus ciprofloxacin, trovafloxacin versus ciprofloxacin and gemifloxacin versus trovafloxacin are shown in Tables 31–33, respectively. The percentages of resistant strains when different breakpoints were considered are shown in Table 34.

Using a breakpoint of ≥4 mg/L for ciprofloxacin resistance, a resistance incidence of 3.5% was found among *S. pneumoniae* respiratory tract isolates in Spain in 1996–97. None of the strains exhibited a gemifloxacin MIC ≥0.5 mg/L, whereas 35.9% had a trovafloxacin MIC ≥0.5 mg/L. Using increasing breakpoints (≥1, ≥2 and ≥4 mg/L), the trovafloxacin resistance rates were 17.9%, 12.8% and 2.5%, respectively, while gemifloxacin resistance rates remained at 0%. None of the ciprofloxacin-resistant strains tested had a gemifloxacin MIC higher than 0.25 mg/L. Gemifloxacin was more active than trovafloxacin and ciprofloxacin against the *S. pneumoniae* isolates; gemifloxacin had an MIC_{90} 33-fold lower than that of trovafloxacin and 133-fold lower than ciprofloxacin.

Gemifloxacin offers a preferred therapeutic alternative in an environment of penicillin/macrolide resistance combined with emerging ciprofloxacin resistance.

Table 31. Comparison of the *in Vitro* Susceptibility of Gemifloxacin Versus Ciprofloxacin (Number of Strains at a Given MIC)

Gemifloxacin	Ciprofloxacin MIC (mg/L)			
MIC (mg/L)	4	8	16	Total
0.007	1	–	–	1
0.015	14	2	–	16
0.03	8	4	–	12
0.06	4	1	1	6
0.12	1	–	1	2
0.25	–	–	2	2
Total	28	7	4	39

Table 32. Comparison of the *in Vitro* Susceptibility of Trovafloxacin Versus Ciprofloxacin (Number of Strains at a Given MIC)

Trovafloxacin	Ciprofloxacin MIC (mg/L)			
MIC (mg/L)	4	8	16	Total
0.03	2	–	–	2
0.06	6	2	–	8
0.12	7	1	–	8
0.25	5	2	–	7
0.5	5	2	–	7
1	1	–	1	2
2	1	–	3	4
4	1	–	–	1
Total	28	7	4	39

Table 33. Comparison of the *in Vitro* Susceptibility of Gemifloxacin Versus Trovafloxacin (Number of Strains at a Given MIC)

Gemifloxacin	Trovafloxacin MIC (mg/L)								
MIC (mg/L)	0.03	0.06	0.12	0.25	0.5	1	2	4	Total
0.007	1	–	–	–	–	–	–	–	1
0.15	1	6	4	3	2	–	–	–	16
0.03	–	1	4	3	1	1	1	1	12
0.06	–	1	–	1	3	1	–	–	6
0.12	–	–	–	–	1	–	1	–	2
0.25	–	–	–	–	–	–	2	–	2
Total	2	8	8	7	7	2	4	1	39

Table 34. Susceptibility of 39 Strains of *S. pneumoniae* with a Ciprofloxacin MIC ≥4 mg/L

Antimicrobial	% strains with						
	MIC_{50}	MIC_{90}	MIC range	MIC ≥0.5	MIC ≥1	MIC ≥2	MIC ≥4
Ciprofloxacin	4	8	4–≥16	100	100	100	100
Trovafloxacin	0.25	2	0.03–4	35.9	17.9	12.8	2.5
Gemifloxacin	0.03	0.06	0.03–0.25	0	0	0	0

The invention provides a method for modulating metabolism of ciprofloxacin-resistant or trovafloxacin-resistant pathogenic bacteria. Skilled artisans can readily choose ciprofloxacin-resistant or trovafloxacin-resistant pathogenic bacteria or patients infected with or suspected to be infected with these organisms to practice the methods of the invention. Alternatively, the bacteria useful in the methods of the invention may be those described herein.

Also provided by the invention is a method of treating or preventing a bacterial infection by ciprofloxacin-resistant or trovafloxacin-resistant pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a quinolone, particularly a gemifloxacin compound to a mammal, preferably a human, suspected of having or being at risk of having an infection with ciprofloxacin-resistant or trovafloxacin-resistant pathogenic bacteria.

While a preferred object of the invention provides a method wherein said ciprofloxacin-resistant or trovafloxacin-resistant pathogenic bacteria is selected from the group consisting of: a bacteria with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a respiratory tract pathogenic bacteria with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a member of the genus *Streptococcus* with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a *Streptococcus pneumoniae* strain with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a penicillin-resistant member of the genus *Streptococcus* with elevated MICs

to or otherwise resistant to ciprofloxacin or trovafloxacin, and a penicillin-resistant *Streptococcus pneumoniae* strain with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin. Other ciprofloxacin-resistant or trovafloxacin-resistant pathogenic bacteria may also be included in the methods. The skilled artisan may identify these organisms as provided herein as well as using other methods known in the art, *e.g.* MIC tests.

The contacting step in any of the methods of the invention may be performed in many ways that will be readily apparent to the skilled artisan. However, it is preferred that the contacting step is a provision of a composition comprising a gemifloxacin compound to a human patient in need of such composition or directly to bacteria in culture medium or buffer.

For example, when contacting a human patient or contacting said bacteria in a human patient or *in vitro*, particularly in any of the methods of the invention, the compositions comprising a quinolone, particularly a gemifloxacin compound, preferably pharmaceutical compositions may be administered in any effective, convenient manner including, for instance, administration by topical, oral, anal, vaginal, intravenous, intraperitoneal, intramuscular, subcutaneous, intranasal or intradermal routes among others.

It is also preferred that these compositions be employed, in any of the methods of the invention, in combination with a non-sterile or sterile carrier or carriers for use with cells, tissues or organisms, such as a pharmaceutical carrier suitable for administration to a subject. Such compositions comprise, for instance, a media additive or a therapeutically effective amount of a compound of the invention, a quinolone, preferably a gemifloxacin compound, and a pharmaceutically acceptable carrier or excipient. Such carriers may include, but are not limited to, saline, buffered saline, dextrose, water, glycerol, ethanol and combinations thereof. The formulation should suit the mode of administration.

Quinolone compounds, particularly gemifloxacin compounds and compositions of the methods of the invention may be employed, in any of the methods of the invention, alone or in conjunction with other compounds, such as bacterial efflux pump inhibitor compounds or antibiotic compounds, particularly non-quinolone compounds, *e.g.*, beta-lactam antibiotic compounds.

In therapy or as a prophylactic, the active agent of a method of the invention is preferably administered to an individual as an injectable composition, for example as a sterile aqueous dispersion, preferably an isotonic one.

Alternatively, the gemifloxacin compounds or compositions in any of the methods of the invention may be formulated for topical application for example in the form of ointments, creams, lotions, eye ointments, eye drops, ear drops, mouthwash, impregnated dressings and sutures and aerosols, and may contain appropriate conventional additives, including, for example, preservatives, solvents to assist drug penetration, and emollients in ointments and creams. Such topical formulations may also contain compatible conventional carriers, for example cream or ointment bases, and ethanol or oleyl alcohol for lotions. Such carriers may constitute from about 1% to about 98% by weight of the formulation; more usually they will constitute up to about 80% by weight of the formulation.

For administration to mammals, and particularly humans, it is expected that the antibacterially effective amount is a daily dosage level of the active agent from 0.001 mg/kg to 10 mg/kg, typically around 0.1 mg/kg to 1 mg/kg, preferably about 1 mg/kg. A physician, in any event, will determine an actual dosage that is most suitable for an individual and will vary with the age, weight and response of the particular individual. The above dosages are exemplary of the average case. There can, of course, be individual instances where higher or lower dosage ranges are merited, and such are within the scope of this invention. It is preferred that the dosage is selected to modulate metabolism of the bacteria in such a way as to inhibit or stop growth of said bacteria or by killing said bacteria. The skilled artisan may identify this amount as provided herein as well as using other methods known in the art, *e.g.* by the application MIC tests.

A further embodiment of the invention provides for the contacting step of the methods to further comprise contacting an in-dwelling device in a patient. In-dwelling devices include, but are not limited to, surgical implants, prosthetic devices and catheters, i.e., devices that are introduced to the body of an individual and remain in position for an extended time. Such devices include, for example, artificial joints, heart valves, pacemakers, vascular grafts, vascular catheters, cerebrospinal fluid shunts, urinary catheters, and continuous ambulatory peritoneal dialysis (CAPD) catheters.

A quinolone, particularly a gemifloxacin compound or composition of the invention may be administered by injection to achieve a systemic effect against a bacteria of the invention, shortly before insertion of an in-dwelling device. Treatment may be continued after surgery during the in-body time of the device. In addition, the composition could also

be used to broaden perioperative cover for any surgical technique to prevent bacterial wound infections.

In addition to the therapy described above, a gemifloxacin compound or composition used in the methods of this invention may be used generally as a wound treatment agent to prevent adhesion of bacteria to matrix proteins, exposed in wound tissue and for prophylactic use in dental treatment as an alternative to, or in conjunction with, antibiotic prophylaxis.

Alternatively, a quinolone, particularly a gemifloxacin compound or composition of the invention may be used to bathe an indwelling device immediately before insertion. The active agent will preferably be present at a concentration of 1μg/ml to 10mg/ml for bathing of wounds or indwelling devices.

Preferred embodiments of the invention include, among other things, methods wherein said composition comprises gemifloxacin, or a pharmaceutically acceptable derivative thereof.

EXAMPLES

The present invention is further described by the following examples. The examples are provided solely to illustrate the invention by reference to specific embodiments. This exemplification's, while illustrating certain specific aspects of the invention, do not portray the limitations or circumscribe the scope of the disclosed invention.

All examples were carried out using standard techniques, which are well known and routine to those of skill in the art, except where otherwise described in detail.

All parts or amounts set out in the following examples are by weight, unless otherwise specified.

Example 1 Bacterial Strains

Test strains of maxillary sinus bacteria were obtained from recent maxillary sinus aspirations. Identification of organisms was by standard methods (see, for example, Murray, P.R., et al. *Manual of Clinical Microbiology.* 6th ed. American Society of Microbiology 1995: 282–620).

Example 2 Antimicrobial Activity Testing

Antimicrobial activity againat maxillary sinus bacteria was tested against 250 selected isolates (Table 1). Emphasis was placed on testing commonly isolated sinusitis organisms or organisms that have demonstrated resistance to common oral therapy.

Example 3 Susceptibility Testing

The agar dilution method using replicate plating of the maxillary sinus organisms onto a series of agar plates of increasing concentrations was used (see, for example, National Committee for Clinical Laboratory Standards. Methods for antimicrobial susceptibility tests for bacteria that growth aerobically. Approved standards M 7-A4. National Committee for Laboratory Standards, Villanova, PA, 1997).

MICs were determined by using doubling dilutions of between 0.02–256 mg/L with an inoculum of 10^4 CFU in area of 5–8 mm.

Mueller–Hinton agar was used for routine susceptibility testing of aerobic and facultative anaerobic bacteria and was supplemented with 5% defibrinated sheep blood for testing those organisms that do not grow on the unsupplemented medium. Haemophilus Test Medium was used for *Haemophilus* spp. and Wilkins–Chalgren agar was used for anaerobes. After incubation at 35°C for 24 h in an aerobic atmosphere for aerobes or facultative anaerobes, in 5–7% CO_2 for *Haemophilus* and in an anaerobic atmosphere for anaerobes, the MIC was determined as the lowest concentration of antimicrobial that completely inhibited growth.

Example 4 Bacterial Strains

The strains were previously isolated from human clinical specimens from a variety of sources, and were identified by standard criteria (Alexander, et al., *J. Clin. Microbiol.*, 35:406-411, 1997; Citron, et al., *Clin. Infect. Dis.*, 23 (Suppl. 1): 78-82, 1996; Holdeman, et al., *Anaerobic Laboratory Manual*, 4th Edition, 1977; Summanen, et al., *Wadsworth Anaerobic Bacteriology Manual*, 5th Edition, 1993). Almost all these isolates were different from those strains used in our prior study (Goldstein, et al., *Antimicrob. Agents Chemother.*, Submitted] when we used the same genus and species. *Bacteroides fragilis* ATCC 25285, and *Bacteroides thetaiotaomicron* ATCC 29741 were tested simultaneously as control strains. The numbers and species of isolates tested are given in Table 6.

Example 5 Compounds

Frozen cultures as described in Example 4 were transferred at least twice on Brucella agar supplemented with hemin, vitamin K_1, and 5% sheep blood to ensure purity and good growth. Susceptibility testing was performed according to National Committee for Clinical Laboratory Standards (NCCLS) standards (*National Committee for Clinical*

Laboratory Standards, 4th Edition, 1997). Brucella agar supplemented with hemin, vitamin K_1, and 5% laked sheep blood was the basal medium used. For *Bilophila wadsworthia*, the agar was also supplemented with pyruvate. Antimicrobial agents were reconstituted according to the manufacturers' instructions. Serial twofold dilutions of antimicrobial agents were prepared on the day of the test and added to the media in varying concentrations (ցg/ml).

The agar plates were inoculated with a Steers replicator (Craft Machine Inc., Chester, PA). The inoculum used was 10^5 CFU per spot. Control plates without antimicrobial agents were inoculated before and after each set of drug-containing plates. The MIC was defined as the lowest concentration of an agent that yielded no growth, or a marked change in the appearance of growth as compared to the growth control plate.

Example 6 Bacterial Strains

A variety of *Legionella* were isolated from respiratory tract and environmental sources. Identification of organisms was by standard methods know in the art (for example, see, Washington, C.W. Jr. *Legionella*. In: Murray *et al.*, eds. *Manual of Clinical Microbiology*. 6th ed. American Society of Microbiology 1995: 533–544).

Example 7 Susceptibility Testing

MICs for *Legionella* strains were determined by standard 2-fold agar dilution procedure using Buffered Yeast Extract agar (herein "BYE") (National Committee for Clinical Laboratory Standards: Methods for antimicrobial susceptibility tests for bacteria that growth aerobically, approved standards M 7-A4. National Committee for Laboratory Standards, Villanova, PA, 1997).

A final innoculum of about 10^4 colony forming units (herein "CFU") was inoculated onto the BYE containing doubling dilutions of antibiotics (0.004–256 mg/L). Plates were incubated at 35°C for 48 hours. An MIC was defined as the lowest concentration of antimicrobial that completely inhibited visible growth. Strains of *Pseudomonas aeruginosa* ATCC 27853 and *L. pneumophila* ATCC 33152 were included as controls.

Example 8 Determination of PAE

The *in vitro* method using the broth technique (Craig, W.A. *Antibiotics in laboratory medicine*. Williams & Wilkins 1986: 515–536) was used to determine the PAE

with Buffered Yeast extract (BYE). Each strain of Example 6 was exposed to antimicrobial concentration of four times the MIC. Fresh inoculum (1 ml, final concentration of 10^6–10^7 CFU/ml) was added to 9 ml of prepared antimicrobial containing medium and to 9 ml of drug-free control medium and incubated at 37°C for 1–2 hours. Antimicrobial agent was removed by three consecutive centrifugations at 1200 x g for 10 minutes. Counts of CFU/ml were performed on all cultures at time zero, before and after washing, and every 1 hour until turbidity develops. The counts of CFU/ml were graphed and the duration of PAE was calculated by equation:

$$PAE = T - C$$

where T is the time required for the count of CFU in the test culture to increase 1 $\log_{10}$ above the count observed immediately after drug removal, and C is the time required for the count of untreated control culture to increase by 1 $\log_{10}$ above the count observed immediately after the completion of the same procedure used on the test culture for drug removal.

Example 9 Bacteria

For agar dilution MICs, quinolone susceptible pneumococci comprised 64 penicillin susceptible (MICs ≤0.06 μg/ml), 68 penicillin intermediate (MICs 0.125-1.0 μg/ml) and 75 penicillin resistant (MIC 2.0-16.0 μg/ml) strains (all quinolone susceptible, with ciprofloxacin MICs ≤4.0 μg/ml). All susceptible, and some intermediate and resistant strains, were recent U.S. isolates. The remainder of intermediate and resistant strains were isolated in South Africa, Spain, France, Central and Eastern Europe, and Korea. Additionally, 28 strains with ciprofloxacin MICs ≥8 μg/ml some from a collection of organisms were tested by agar dilution. Additionally these strains were tested for mutations in parC, gyrA, parE, and gyrB (Pan, et al., *Antimicrob. Agents Chemother.* 40:2321-2326, 1996) and for efflux mechanism (Brenwald, et al., *Antimicrob. Agents Chemother.* 42:2032-2035, 1998). For time-kill studies, 4 penicillin susceptible, 4 intermediate and 4 resistant strains (2 quinolone resistant) were tested, while for PAE studies 5 quinolone susceptible and 1 resistant strains were studied.

Example 10 Antimicrobials and MIC testing.

Agar dilution methodology was performed on the 234 strains of Example 9 as described previously (M.R. Jacobs, *Clin. Infect. Dis.* 15:119-127, 1992; Jacobs, et al., *Rev. Med. Microbiol.* 6:77-93, 1995), using Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md) supplemented with 5% sheep blood. Broth MICs for 12 strains tested by

time-kill and 6 tested by PAE were performed according to NCCLS recommendations (*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically*, 3rd Edition, NCCLS, Villanova, PA) using cation-adjusted Mueller-Hinton broth with 5% lysed defibrinated horse blood. Standard quality control strains, including *Streptococcus pneumoniae* ATCC 49619, were included in each run of agar and broth dilution MICs.

Example 11 Time-kill testing

For time-kill studies of the organisms in Example 9, glass tubes containing 5 ml cation-adjusted Mueller-Hinton broth (Difco) + 5% lysed horse blood with doubling antibiotic concentrations were inoculated with 5 x 10^5 to 5 x 10^6 cfu/ml and incubated at 35°C in a shaking water bath. Antibiotic concentrations were chosen to comprise 3 doubling dilutions above and 3 dilutions below the agar dilution MIC. Growth controls with inoculum but no antibiotic were included with each experiment (Pankuch, et al., *Antimicrob. Agents Chemother.* 38:2065-2072, 1994; Pankuch, et al., *Antimicrob. Agents Chemother.* 40:1653-1656, 1996).

Lysed horse blood was prepared as described previously. The bacterial inoculum was prepared by diluting a 16 h broth (medium as above) culture in the same medium. Dilutions required to obtain the correct inoculum (5 x 10^5-5 x 10^6 cfu/ml) were determined by prior viability studies using each strain (Pankuch, et al., *Antimicrob. Agents Chemother.* 38:2065-2072, 1994; Pankuch, et al., *Antimicrob. Agents Chemother.* 40:1653-1656, 1996).

To inoculate each tube of serially diluted antibiotic, 50 ìl of diluted inoculum was delivered by pipette beneath the surface of the broth. Tubes were then vortexed and plated for viability counts within 10 min (approximately 0.2 h). The original inoculum was determined by using the untreated growth control. Only tubes containing an initial inoculum within the range of 5 x 10^5 to 5 x 10^6 cfu/ml were acceptable (Pankuch, et al., *Antimicrob. Agents Chemother.* 38:2065-2072, 1994; Pankuch, et al., *Antimicrob. Agents Chemother.* 40:1653-1656, 1996).

Viability counts of antibiotic-containing suspensions were performed by plating ten-fold dilutions of 0.1 ml aliquots from each tube in sterile Mueller-Hinton broth onto trypticase soy agar 5% sheep blood agar plates (BBL). Recovery plates were incubated for up to 72 h. Colony counts were performed on plates yielding 30-300 colonies. The lower limit of sensitivity of colony counts was 300 cfu/ml (Pankuch, et al., *Antimicrob. Agents Chemother.* 38:2065-2072, 1994; Pankuch, et al., *Antimicrob. Agents Chemother.* 40:1653-1656, 1996).

Time-kill assays were analysed by determining the number of strains which yielded a Älog$_{10}$ cfu/ml of -1, -2 and -3 at 0, 3, 6, 12 and 24 h, compared to counts at time 0 h. Antimicrobials were considered bactericidal at the lowest concentration that reduced the original inoculum by ≥3 log$_{10}$ cfu/ml (99.9%) at each of the time periods, and bacteriostatic if the inoculum was reduced by 0-3 log$_{10}$ cfu/ml. With the sensitivity threshold and inocula used in these studies, no problems were encountered in delineating 99.9% killing, when present. The problem of bacterial carryover was addressed as described previously. For macrolide time-kill testing, only strains with MICs ≤4.0 µg/ml were tested (Pankuch, et al., *Antimicrob. Agents Chemother.* 38:2065-2072, 1994; Pankuch, et al., *Antimicrob. Agents Chemother.* 40:1653-1656, 1996).

Example 12 Post-antibiotic effect testing.

The post-antibiotic effect (PAE) (Craig, et al., *V. Lorian (ed.) Antibiotics in Laboratory Medicine,* Williams and Wilkins, Baltimore, pages 296-329, 1996) was determined for the organisms of Example 9 by the viable plate count method, using Mueller-Hinton broth (MHB) supplemented with 5% lysed horse blood when testing pneumococci. The PAE was induced by exposure to 10 x MIC for 1 h (Craig, et al., *V. Lorian (ed.) Antibiotics in Laboratory Medicine,* Williams and Wilkins, Baltimore, pages 296-329, 1996; Spangler, et al., *Antimicrob. Agents Chemother.* 41:2173-2176, 1997; Spangler, et al., *Antimicrob. Agents Chemother.* 42:1253-1255, 1998). Additionally, the one quinolone resistant strain was exposed at quinolone concentrations 5 x MIC. Tubes containing 5 ml broth with antibiotic were inoculated with approximately 5 x 10^6 cfu/ml. Growth controls with inoculum but no antibiotic were included with each experiment. Tubes were placed in a shaking water bath at 35°C for 1 h. At the end of the exposure period, cultures were diluted 1:1000 to remove antibiotic. A control containing bacteria pre-exposed to antibiotic at a concentration of 0.01 x MIC was also prepared (Spangler, et al., *Antimicrob. Agents Chemother.* 41:2173-2176, 1997; Spangler, et al., *Antimicrob. Agents Chemother.* 42:1253-1255, 1998).

Viability counts were determined before exposure and immediately after dilution (0 h), and then every 2 h until tube turbidity reached a #1 McFarland standard. Inocula were prepared by suspending growth from an overnight blood agar plate in broth. The broth was incubated at 35°C for 2-4 h in a shaking water bath until turbidity matched a #1 McFarland standard, and checked for viability by plate counts (Spangler, et al., *Antimicrob. Agents*

Chemother. 41:2173-2176, 1997; Spangler, et al., *Antimicrob. Agents Chemother.* 42:1253-1255, 1998).

The PAE was defined as PAE = T-C; T = time required for viability counts of an antibiotic-exposed culture to increase by 1 $\log_{10}$ above counts immediately after dilution; C = corresponding time for growth control. For each experiment, viability counts ($\log_{10}$ cfu/ml) were plotted against time, and results expressed as the mean of two separate assays ± SD (Craig, et al., *V. Lorian (ed.), Antibiotics in Laboratory Medicine,* Williams and Wilkins, Baltimore, pages 296-329, 1996).

Example 13 PCR of Quinolone Resistance Determinants and DNA Sequence Analysis.

Polymerase chain reaction method (PCR) was used to amplify parC, parE, gyrA,and gyrB of the organisms of Example 9 using primers and cycling conditions described by Pan and Fisher (Pan, et al., *Antimicrob. Agents Chemother.* 40:2321-2326, 1996). Template DNA for PCR was prepared using Prep-A-Gene kit (Bio-Rad, Hercules, CA) as recommended by the manufacturer. After amplification PCR products were purified from excess primers and nucleotides using QIAquick PCR Purification kit as recommended by the manufacturer (Qiagen, Valencia, CA) and sequenced directly using Applied Biosystems Model 373A DNA sequencer. Strains with mutations widely described in the literature (e.g. Ser79-Tyr or Phe in ParC and Ser83-Tyr or Phe in GyrA) were sequenced once in the forward direction. Strains with no mutations in any of the above mentioned genes or with a previously undescribed mutation were sequenced twice in the forward direction and once in the reverse direction on products of independent PCR reactions (Davies, et al., *Antimicrob. Agents Chemother.* 43:1177-1182, 1999).

Example 14 Determination of Efflux Mechanism.

MICs for the orgainsms of Example 9 were determined in the presence and absence of 10 µg/ml of reserpine (Sigma Chemicals, St. Louis, MO) as known in the art. Strains with at least a twofold lower ciprofloxacin MIC in the presence of reserpine were then tested against the other quinolones in the presence of reserpine. Results were repeated three times (Brenwald, et al., *Antimicrob. Agents Chemother.* 42:2032-2035, 1998; Davies, et al., *Antimicrob. Agents Chemother.* 43:1177-1182, 1999).

Example 15 Bacterial strains

28 strains with ciprofloxacin MICs ≥8 μg/ml were tested by agar dilution. Additionally these strains were tested for mutations in *parC*, *gyrA*, *parE*, and *gyrB* (Pan, et al., *Antimicrob. Agents Chemother.* 40:2321-2326, 1996) and for efflux mechanism (Brenwald, et al., *Antimicrob. Agents Chemother.* 42:2032-2035, 1998).

Example 16 Antimicrobials and MIC Testing

Gemifloxacin susceptibility powder was obtained from SmithKline Beecham Laboratories, Harlow, UK. Agar dilution methodology was performed on 28 strains as described previously (M.R. Jacobs, *Clin. Infect. Dis.* 15:119-127, 1992 and M.R. Jacobs, *Rev. Med. Microbiol.* 6:77-93, 1995), using Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md) supplemented with 5% sheep blood. Standard quality control strains, including *Streptococcus pneumoniae* ATCC 49619, were included in each run of agar dilution MICs.

Example 17 PCR of Quinolone Resistance Determinants and DNA Sequence Analysis

PCR was used to amplify *parC*, *parE*, *gyrA* and *gyrB* of the orgainsms of Example 15 using primers and cycling conditions described by Pan *et al* (Pan et al., *Antimicrob. Agents Chemother.* 40:2321-2326, 1996). Template DNA for PCR was prepared using Prep-A-Gene kit (Bio-Rad, Hercules, CA, USA) as recommended by the manufacturer. After amplification PCR products were purified from excess primers and nucleotides using QIAquick PCR Purification kit as recommended by the manufacturer (Qiagen, Valencia, CA, USA) and sequenced directly using Applied Biosystems Model 373A DNA sequencer.

Example 18 Determination of Efflux Mechanism

MICs were determined of the orgainsms of Example 15 in the presence and absence of 10 μg/ml of reserpine (Sigma Chemicals, St. Louis, MO, USA) as described previously (Brenwald, et al., *Antimicrob. Agents Chemother.* 42:2032-2035, 1998 and Davies, et al., *Antimicrob. Agents Chemother.* 43:1177-1182, 1999). Strains with at least a twofold lower ciprofloxacin MIC in the presence of reserpine were then tested against the other quinolones in the presence of reserpine. Results were repeated three times previously (Brenwald, et al., *Antimicrob. Agents Chemother.* 42:2032-2035, 1998 and Davies, et al., *Antimicrob. Agents Chemother.* 43:1177-1182, 1999).

Example 19 MIC Determination

Inocula were prepared from chocolate agar plates incubated for a full 24 hours by the direct colony suspension method as follows: In a tube of Mueller-Hinton broth (Difco), an organism suspension was made to a density of a 0.5 McFarland standard (1 x 10^8 CFU/ml). The latter inoculum was diluted in sterile saline such that final organisms suspensions in trays yielded colony counts of 3–8 x 10^5 CFU/ml. Frozen microdilution trays were obtained from MicroMedia Systems, Inc. (Cleveland, OH, USA). Each tray contained all antimicrobials prepared in freshly made HTM. Wells were inoculated with 100 μl suspensions and incubated in ambient air at 35°C for 20–24 hours. The lowest drug concentration showing no growth was read as the MIC. Standard quality control strains, including *H. influenzae* ATCC 49766, *H. influenzae* ATCC 49247, *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were included with each run.

Example 20 PCR and DNA Sequencing of Quinolone-resistant Determining Region of *parC*, *parE*, *gyrA*, and *gyrB*

From the organisms of Example 19, template DNA for PCR was prepared as follows: a colony from overnight growth was lysed by incubation for 1 hour at 37°C in lysis buffer (6 mM Tris-HCL [pH 7.4], 1 M NaCl, 10 mM EDTA [pH 8.0], 0.2% deoxycholate, 0.5% sodium lauroyl sarcosine) to which lysozyme (Sigma, St. Louis, MO, USA) at 0.5 mg/ml and lysostaphin (Sigma) at 0.05 mg/ml were added fresh. DNA was isolated from the lysed cells using a Prep-A-Gene kit (Bio-Rad, Hercules, CA, USA) as recommended by the manufacturer. PCR was carried out in a final volume of 100 μl containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 200 μM each dNTPS, 5 pmol of each primer, 5–10 ng DNA template, and 2.5 U Taq DNA polymerase (Fisher Biotech). Conditions for PCR were 30 cycles of 94°C for 1 minute, annealing at 53°C for 1 minute, and extension at 72°C from 3 minutes. For *parC* a 370 bp region encoding residues 41 to 163 was amplified using primers HFPARCUP (5'-TGGTTTAAAACCCGTTCA-3', nucleotide positions 120 to 137) and HFPARCDN (5'-AGCAGGTAAATATTGTGG-3', positions 473–490). For *parE* a 471 bp region encoding residues 335 to 491 was amplified using primers HFPAREUP (5'-GAACGCTTATCATCACGCCA-3', positions 1003 to 1022) and HFPAREDN (5'-AGCATCCGCGAGAATACAGA-3', positions 1454 to 1473). For *gyrA* a 375 bp region encoding residues 47 to 171 was amplified using primers HFGYRAUP (5'-

CCGCCGCGTACTGTTCT-3', positions 138 to 154) and HFGYRADN (5'-CCATTTGCTAAAAGTGC-3', positions 496 to 512). For *gyrB* a 445 bp region encoding residues 367 to 513 was amplified using primers HFGYRBFOR (5'-GGAAAATCCTGCAGATGC-3', positions 1095 to 1113) and HFGYRBBAC (5'-AAGCAACGTACGGATGTG-3', positions 1522 to 1539). After amplification PCR products were purified from excess primers and nucleotides using a QIAquick PCR purification kit (Qiagen, Valencia, CA, USA) and sequenced directly by using an Applied Biosystems model 373A DNA sequencer. All genes were sequenced twice in the forward and reverse directions on products of independent PCRs.

Example 21 Isolates and Antimicrobial Agents

Of 200 recently clinically isolated strains of pneumococci, 68 were penicillin-susceptible (MIC <1.0 μg/ml); 67 were penicillin intermediate (MIC 0.1–1.0 μg/ml) and 65 penicillin resistant (MIC ≥2.0 μg/ml). The 200 strains included 39 with raised quinolone MICs (ciprofloxacin MICs ≥8 μg/ml – 21 penicillin susceptible, 12 intermediate, 6 penicillin resistant). Cultures were maintained at -70°C in double-strength skim milk (Difco Laboratories, Detroit, MI). Gemifloxacin susceptibility powder, disks and E-tests (AB Biodisk, Solna, Sweden) were obtained from SmithKline Beecham Laboratories, Collegeville, PA, USA.

Example 22 Agar Dilution MICs

MICs for the organisms of Example 21 were performed (Jacobs, et al., *Clin. Infect. Dis.* 15:119-127, 1992 and Clark, et al., *J. Clin. Microbiol.* 36:3579-3584, 1998) on Mueller-Hinton agar supplemented with 5% sheep blood, incorporating compounds at concentrations from 0.002–8 μg/ml in doubling dilutions. Inocula were prepared by suspending growth from overnight cultures in Mueller-Hinton broth to a turbidity of a 0.5 McFarland standard. Final inocula contained 10^4 organisms/spot. Plates were inoculated with a Steers replicator with 3 mm inoculating pins, and incubated overnight at 35°C in ambient air. The lowest concentration of antibiotic showing no growth was read as the MIC. Quality control strains – *Staphylococcus aureus* ATCC 29213 and *Streptococcus pneumoniae* ATCC 49619 -- were included in each run.

Example 23 Microdilution MICs

MICs for the organisms of Example 21 were determined by the method recommended by the NCCLS, (*Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically,* 4th Edition, NCCLS, Villanova, PA, 1997) using cation-adjusted Mueller-Hinton broth (Difco Laboratories) supplemented with 5% lysed defibrinated horse blood. Trays were prepared in-house. Suspensions with a turbidity equivalent to that of a 0.5 McFarland standard were prepared by suspending growth from blood agar plates in 2 ml Mueller-Hinton broth. Suspensions were further diluted 1:10 to obtain a final inoculum (10 μL) containing 5 x 10^5 CFU/mL. Trays were incubated 20–24 hours in ambient air at 35°C. Standard quality control strains (as above) were included in each run.

Example 24 E-test MICs

Standard methodology was used to obtain MICs for the organisms of Example 21 (Clark, et al., *J. Clin. Microbiol.* 36:3579-3584, 1998). Mueller-Hinton plates supplemented with 5% sheep blood (BBL Microbiology Systems, Cockeysville, Md, USA) were inoculated with a 0.5 McFarland suspension harvested from plates, and E-test strips (AB Biodisk, Solna, Sweden) placed on each. After overnight incubation at 35°C, the MIC was read as the intersect where the ellipse of growth inhibition intersects the strip. E-test MICs were performed both in air and in CO_2. E-test MICs were rounded up to the next highest doubling dilution.

Example 25 Disk Diffusion

This was by standard NCCLS methodology (*Performance Standards for Antimicrobial Disk Susceptibility Tests,* 6th Edition, NCCLS, Villanova, PA, 1997) using 5 μg gemifloxacin disks (BBL) and Mueller-Hinton plates supplemented with 5% sheep blood (BBL), inoculated with a 0.5 McFarland suspension. After overnight incubation in both air and 5% CO_2 at 35°C, zone diameters were measured with calipers.

Each reference cited herein is hereby incorporated by reference in its entirety. Moreover, each patent application to which this application claims priority is hereby incorporated by reference in its entirety.

Example 26 Bacterial Strains

In a nationwide surveillance study consisting of 14 geographically selected centres in Spain (Fenoll, A.; Burgon, C.M.; Muñoz, R; Vicioso, D. *et al.* Serotype distribution and antimicrobial resistance of *Streptococcus pneumoniae* isolates causing systemic infections in Spain, 1979–1989. *Rev Infect Dis* 1991; **13:** 56–60), 1113 *S. pneumoniae* isolates were collected from patients with community-acquired respiratory tract infections in the period May 1996–April 1997 (Baquero, F.; García-Rodríguez, J.A.; García de Lomas, J.; Aguilar, L. *et al.* Antimicrobial resistance of 1113 *Streptococcus pneumoniae* isolates from patients with respiratory tract infections in Spain: results of a 1-year (1996–1997) multicenter surveillance study. *Antimicrob Agents Chemother* 1999; **43:** 357–359; Liñares, J., Pallarés, R.; Alonso, T.; Pérez, J.L. *et al.* Trends in antimicrobial resistance of clinical isolates of *Streptococcus pneumoniae* in Bellvitge Hospital, Barcelona, Spain (1979–1990). *Clin Infect Dis* 1992; **15:** 99–105). Susceptibility testing was performed using a semi-automated microdilution method in Todd–Hewitt broth following National Committee for Clinical Laboratory Standards (NCCLS) guidelines (National Committee for Clinical Laboratory Standards 1997. Approved Standard M7-A4 Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically (fourth edition). NCCLS, Wayne, PA, USA), with a final inoculum of 5 x 10^5 CFU/ml. Cultures were incubated for 24 h at 35°C. Strains with a ciprofloxacin MIC ≥4 mg/L were tested *in vitro* for gemifloxacin and trovafloxacin susceptibility in triplicate following the same methodology. Modal values were calculated.

Each reference cited herein is hereby incorporated by reference in its entirety. Moreover, each patent application to which this application claims priority is hereby incorporated by reference in its entirety.

What is claimed is:

1. A method for modulating metabolism of pneumococcal pathogenic bacteria comprising the step of contacting pneumococcal pathogenic bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof.

2. A method of treating or preventing a bacterial infection by pneumococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof, to a mammal suspected of having or being at risk of having an infection with pneumococcal pathogenic bacteria.

3. The method of claim 1 or 2 wherein said pneumococcal pathogenic bacteria is selected from the group consisting of:

bacteria comprising a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB; bacteria comprising a mutation in ParC at S79-F or Y, D83-N, R95-C, or K137-N; bacteria comprising a mutation in gyrA at S83-A, C, F, or Y; E87-K; or S116-G; bacteria comprising a mutation in parE at D435-N or I460-V; bacteria comprising a mutation in gyrB at D435-N or E474-K; bacteria comprising at least four mutations in a QRDR or parC, gyrA, parE, and gyrB; bacteria comprising a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in ParC at S79-F or Y, D83-N, R95-C, or K137-N; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in gyrA at S83-A, C, F, or Y; E87-K; or S116-G; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in parE at D435-N or I460-V; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in gyrB at D435-N or E474-K;

bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise at least four mutations in a QRDR or parC, gyrA, parE, and gyrB; bacteria that are ciprofloxacin-resistant, levofloxacin-resistant, sparfloxacin-resistant, grepafloxacin-resistant, or trovafloxacin-resistant, or a combination thereof, that comprise a mutation in a quinolone

resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB; *Streptococcus pneumoniae* bacteria comprising a mutation in ParC at S79-F or Y, D83-N, R95-C, or K137-N; *Streptococcus pneumoniae* bacteria comprising a mutation in gyrA at S83-A, C, F, or Y; E87-K; or S116-G; *Streptococcus pneumoniae* bacteria comprising a mutation in parE at D435-N or I460-V; *Streptococcus pneumoniae* bacteria comprising a mutation in gyrB at D435-N or E474-K; *Streptococcus pneumoniae* bacteria comprising at least four mutations in a QRDR or parC, gyrA, parE, and gyrB; and *Streptococcus pneumoniae* bacteria comprising a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA, parE, and/or gyrB.

4. A method for modulating the activity of a topoisomerase comprising a mutation in a quinolone resistance-determining region (QRDR) of parC, gyrA or parE or gyrB.

5. The method of claim 4 wherein said mutation in ParC is at S79-F or Y, D83-N, R95-C, or K137-N; said mutation in gyrA is at S83-A, C, F, or Y; E87-K; or S116-G; said mutation in parE is at D435-N or I460-V; or said mutation in gyrB is at D435-N or E474-K.

6. A method for modulating metabolism of quinolone-resistant pneumococcal pathogenic bacteria comprising the step of contacting quinolone-resistant pneumococcal pathogenic bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof.

7. A method of treating or preventing a bacterial infection by quinolone-resistant pneumococcal pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof, to a mammal suspected of having or being at risk of having an infection with quinolone-resistant pneumococcal pathogenic bacteria.

8. The method of claim 6 or 7 wherein said quinolone-resistant pneumococcal pathogenic bacteria is selected from the group consisting of: a pneumococcal strain comprising a mutation in the quinolone resistance-determining region (QRDR) of *parC* and/or *gyrA*; a pneumococcal strain comprising a mutation in ParC said mutation comprising S79→F and/or Y, D83→G and/or N, N91→D, R95→C, and/or K137→N; a pneumococcal strain comprising a mutation in GyrA said mutation comprising S81→A, C, F, and/or Y; E85→K; and/or S114→G; a pneumococcal strain comprising a mutation in ParE said mutation comprising D435→N and/or I460→V; a pneumococcal strain comprising a mutation in GyrB said mutation comprising D435→N and/or E474→K; a pneumococcal strain comprising a mutation in comprising three or four mutations in a

QRDRs of *parC*, *gyrA*, *parE*, and/or *gyrB*; a pneumococcal strain comprising a mutation in comprising three or four mutations in a QRDRs of *parC*, *gyrA*, *parE*, and/or *gyrB*, any of which are resistant to ciprofloxacin, levofloxacin, or sparfloxacin; and a pneumococcal strain comprising a mutation in comprising three or four mutations in a QRDRs of *parC*, *gyrA*, *parE*, and/or *gyrB*, any of which also comprising an efflux mechanism of quinolone resistance.

9. A method for modulating metabolism of fluoroquinolone resistant pathogenic bacteria comprising the step of contacting fluoroquinolone resistant pathogenic bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof.

10. A method of treating or preventing a bacterial infection by fluoroquinolone resistant pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof, to a mammal suspected of having or being at risk of having an infection with fluoroquinolone resistant pathogenic bacteria.

11. The method of claim 9 or 10 wherein said fluoroquinolone resistant pathogenic bacteria is selected from the group consisting of:

a ciprofloxacin resistant strain of *S. pneumoniae*, *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae*, a trovafloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a fluoroquinolone resistant strain of *S. pneumoniae*, a fluoroquinolone resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, and a fluoroquinolone resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region.

12. A method for modulating metabolism of fluoroquinolone resistant pathogenic bacteria comprising the step of contacting fluoroquinolone resistant pathogenic bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof.

13. A method of treating or preventing a bacterial infection by fluoroquinolone resistant pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof, to a mammal suspected of having or being at risk of having an infection with fluoroquinolone resistant pathogenic bacteria.

14. The method of claim 12 or 13 wherein said fluoroquinolone resistant pathogenic bacteria is selected from the group consisting of: a ciprofloxacin resistant strain of *S. pneumoniae, S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae,* a trovafloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a fluoroquinolone resistant strain of *S. pneumoniae,* a fluoroquinolone resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, and a fluoroquinolone resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region.

15. A method for modulating metabolism of fluoroquinolone resistant pathogenic bacteria comprising the step of contacting fluoroquinolone resistant pathogenic bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, and a ciprofloxicin compound, or antibacterially effective derivatives of either thereof.

16. A method of treating or preventing a bacterial infection by fluoroquinolone resistant pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound and a ciprofloxicin compound, or antibacterially effective derivatives of either thereof, to a mammal suspected of having or being at risk of having an infection with fluoroquinolone resistant pathogenic bacteria.

17. The method of claim 15 or 16 wherein said fluoroquinolone resistant pathogenic bacteria is selected from the group consisting of: a ciprofloxacin resistant strain of *S. pneumoniae, S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a ciprofloxacin resistant strain of *S. pneumoniae* having a DNA gyrase

(*gyrA*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae,* a trovafloxacin resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, a trovafloxacin resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region, a fluoroquinolone resistant strain of *S. pneumoniae,* a fluoroquinolone resistant strain of *S. pneumoniae* having a topisomerase *IV* (*parC*) mutation in the QRDR region, and a fluoroquinolone resistant strain of *S. pneumoniae* having a DNA gyrase (*gyrA*) mutation in the QRDR region.

18. A method for modulating metabolism of fluoroquinolone resistant pathogenic bacteria comprising the step of contacting fluoroquinolone resistant pathogenic bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, followed by a ciprofloxicin compound, or antibacterially effective derivatives of either thereofs

19. A method of treating or preventing a bacterial infection by fluoroquinolone resistant pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound followed by a ciprofloxicin compound, or antibacterially effective derivatives of either thereof,to a mammal suspected of having or being at risk of having an infection with fluoroquinolone resistant pathogenic bacteria.

20. A method for modulating metabolism of ciprofloxacin-resistant and/or ciprofloxicin-sensitive bacteria comprising the step of contacting ciprofloxacin-resistant and/or ciprofloxicin-sensitive bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof.

21. A method of treating or preventing a bacterial infection by ciprofloxacin-resistant and/or ciprofloxicin-sensitive bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof, to a mammal suspected of having or being at risk of having an infection with ciprofloxacin-resistant and/or ciprofloxicin-sensitive bacteria.

22. The method of claim 20 or 21 wherein said ciprofloxacin-resistant and/or ciprofloxicin-sensitive bacteria is selected from the group consisting of:

ciprofloxacin-susceptible pneumococci having an MIC ≤ 4 μg/ml of ciprofloxacin;

ciprofloxacin-resistant pneumococci having an MIC ≤ 8 μg/ml of ciprofloxacin;

ciprofloxacin-susceptible *Streptococcus pneumoniae* having an MIC ≤ 4 μg/ml of ciprofloxacin; and

ciprofloxacin-resistant *Streptococcus pneumoniae* having an MIC ≤8 μg/ml of ciprofloxacin.

23. A method for modulating metabolism of ciprofloxacin-resistant or trovafloxacin-resistant pathogenic bacteria comprising the step of contacting ciprofloxacin-resistant or trovafloxacin-resistant pathogenic bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, or antibacterially effective derivatives thereof.

24. A method of treating or preventing a bacterial infection by ciprofloxacin-resistant or trovafloxacin-resistant pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound to a mammal suspected of having or being at risk of having an infection with ciprofloxacin-resistant or trovafloxacin-resistant pathogenic bacteria.

25. The method of claim 23 or 24 wherein said ciprofloxacin-resistant or trovafloxacin-resistant pathogenic bacteria is selected from the group consisting of:

a bacteria with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a respiratory tract pathogenic bacteria with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a member of the genus *Streptococcus* with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a *Streptococcus pneumoniae* strain with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, a penicillin-resistant member of the genus *Streptococcus* with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin, and a penicillin-resistant *Streptococcus pneumoniae* strain with elevated MICs to or otherwise resistant to ciprofloxacin or trovafloxacin.

26. A method for modulating metabolism of atypical upper respiratory pathogenic bacteria comprising the step of contacting atypical upper respiratory pathogenic bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof.

27. A method of treating or preventing a bacterial infection by atypical upper respiratory pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof, to a mammal suspected of having or being at risk of having an infection with atypical upper respiratory pathogenic bacteria.

28. The method of claim 26 or 27 wherein said atypical upper respiratory pathogenic bacteria is selected from the group consisting of:

a member of the genus *Legionella,* a member of the genus, *Pseudomonas, Pseudomonas aeruginosa* strain, a *L. pneumophila* strain, a *L. pneumophila* serogroup 1, a *L. pneumophila* serogroup 2, a *L. pneumophila* serogroup 3, a *L. pneumophila* serogroup 4, a *L. pneumophila* serogroup 5, a *L. pneumophila* serogroup 6, a *L. pneumophila* serogroup 7, a *L. pneumophila* serogroup 8, a *L. dumoffii* strain, a *L. longbeacheae* strain, a L. micdadei strain, a L. oakridgensis strain, a *L. feelei* strain, a *L. anisa* strain, a *L. sainthelensi* strain, a *L. bozemanii* strain, a *L. gormanii* strain, a *L. wadsworthii* strain, a *L. jordanis*; strain and a *L. gormanii* strain.

29. The method of claim 26 or 27 wherein said contacting or administering is performed once daily.

30. A method for modulating metabolism of maxillary sinus pathogenic bacteria comprising the step of contacting maxillary sinus pathogenic bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof.

31. A method of treating or preventing a bacterial infection by maxillary sinus pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof, to a mammal suspected of having or being at risk of having an infection with maxillary sinus pathogenic bacteria.

32. The method of claim 30 or 31 wherein said maxillary sinus pathogenic bacteria is selected from the group consisting of:

a bacterial strain isolated from acute or chronic maxillary sinusitis; and

a maxillary sinus isolate of *S. aureus, S. pneumoniae, Haemophilus* spp., *M. catarrhalis*, and anaerobic strain or non-fermentative Gram negative bacilli, *Neisseria meningitidis* and β-haemolytic *Streptococcus.*

33. The method of claim 30 or 31 wherein said bacteria is selected from the group consisting of: a bacterial strain isolated from acute or chronic maxillary sinusitis; a maxillary sinus isolate of *Staphylococcus aureus, Streptococcus pneumoniae, Haemophilus* spp., *Moraxella catarrhalis,* an anaerobic strain or non-fermentative Gram negative bacilli, *Neisseria meningitidis,* β-haemolytic *Streptococcus, Haemophilus influenzae,* an *Enterobacteriaceae*, a non-fermentative Gram negative bacilli, *Streptococcus pneumoniae, Streptococcus pyogenes,* a methicillin-resistant *Staphylococcus* spp., *Legionella pneumophila, Mycoplasma* spp. and *Chlamydia* spp., *Haemophilus influenzae,*

Haemophilus parainfluenzae, *Peptostreptococcus*, *Bacteroides* spp., and *Bacteroides urealyticus*.

34. A method for modulating metabolism of pathogenic Mycoplasma bacteria comprising the step of contacting pathogenic Mycoplasma bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof.

35. A method of treating or preventing a bacterial infection by pathogenic Mycoplasma bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof, to a mammal suspected of having or being at risk of having an infection with pathogenic Mycoplasma bacteria.

36. The method of claim 34 or 35 wherein said pathogenic Mycoplasma bacteria is selected from the group consisting of:

Mycoplasma pneumoniae, *M. hominis*, *M. fermentans*, *M. genitalium*, *M. penetrans* and *Ureaplasma urealyticum*.

37. A method for modulating metabolism of anaerobic pathogenic bacteria comprising the step of contacting anaerobic pathogenic bacteria with an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof.

38. A method of treating or preventing a bacterial infection by anaerobic pathogenic bacteria comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof, to a mammal suspected of having or being at risk of having an infection with anaerobic pathogenic bacteria.

39. The method of claim 37 or 38 wherein said anaerobic pathogenic bacteria is selected from the group consisting of:

a member of the genus *Peptostreptococci*, a *Peptostreptococci asaccharolyticus*, a *Peptostreptococci magnus*, a *Peptostreptococci micros*, a *Peptostreptococci prevotii*, a *Porphyromonas asaccharolytica*, a *Porphyromonas canoris*, a *Porphyromonas gingivalis*, a *Porphyromonas macaccae*, a member of the genus *Actinomyces*, an *Actinomyces israelii*, an *Actinomyces odontolyticus*, a member of the genus *Clostridium*, a *Clostridium innocuum*, a *Clostridium clostridioforme*, a *Clostridium difficile*, a member of the genus *Anaerobiospirillum*, a *Bacteroides tectum*, a *Bacteroides ureolyticus*, a *Bacteroides gracilis* (*Campylobacter gracilis*), a *Prevotella intermedia*, a *Prevotella heparinolytica*, a

Prevotella oris-buccae, a *Prevotella bivia,* a *Prevotella melaninogenica,* a member of the genus *Fusobacterium,* a *Fusobacterium naviforme,* a *Fusobacterium necrophorum,* a *Fusobacterium varium,* a *Fusobacterium ulcerans,* a *Fusobacterium russii,* a member of the genus *Bilophila,* a *Bilophila wadsworthia.*

40. A method for modulating metabolism of a rare pathogenic H. influenzae strain comprising the step of contacting a rare pathogenic H. influenzae strain with an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof.

41. A method of treating or preventing a bacterial infection by a rare pathogenic H. influenzae strain comprising the step of administering an antibacterially effective amount of a composition comprising a gemifloxacin compound, or an antibacterially effective derivative thereof, to a mammal suspected of having or being at risk of having an infection with a rare pathogenic H. influenzae strain.

42. The method of claim 40 or 41 wherein said rare pathogenic H. influenzae strain is selected from the group consisting of:

bacteria comprising a mutation set forth in Table 25 or 26; a *Haemophilus influenzae* strain set forth in Table 25 or 26; bacteria of the genus *Haemophilus* comprising a mutation set forth in Table 25 or 26; and bacteria of the species *Haemophilus influenzae* comprising a mutation set forth in Table 25 or 26.

43. The method of any one of claims 1, 3, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 22, 23, 25, 26, 28, 29, 30, 31, 33, 34, 36, 37, 39, 40 or 42 wherein said modulating metabolism is inhibiting growth of said bacteria.

44. The method of any one of claims 1, 3, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 22, 23, 25, 26, 28, 29, 30, 31, 33, 34, 36, 37, 39, 40 or 42 wherein said modulating metabolism is killing said bacteria.

45. The method of any one of claims 1, 3, 6, 8, 9, 11, 12, 14, 15, 17, 18, 20, 22, 23, 25, 26, 28, 29, 30, 31, 33, 34, 36, 37, 39, 40, 42, 43 or 44 wherein said contacting said bacteria comprises the further step of introducing said composition into a mammal.

46. The method of any one of claims 2, 3, 7, 8, 13, 14, 16, 17, 19, 21, 22, 24, 25, 27, 28, 29, 31, 32, 33, 35, 36, 38, 39, 41, 42 or 45 wherein said mammal is a human.

47. The method according to any one of the preceding claims wherein the gemifloxacin compound is gemifloxacin or a pharmaceutically acceptable salt thereof.

48. The method according to claim 47 wherein the gemifloxacin compound is gemifloxacin mesylate or a hydrate thereof.

49. The method according to claims 48 wherein the gemifloxacin compound is gemifloxacin mesylate sesquihydrate.

Figure 1.

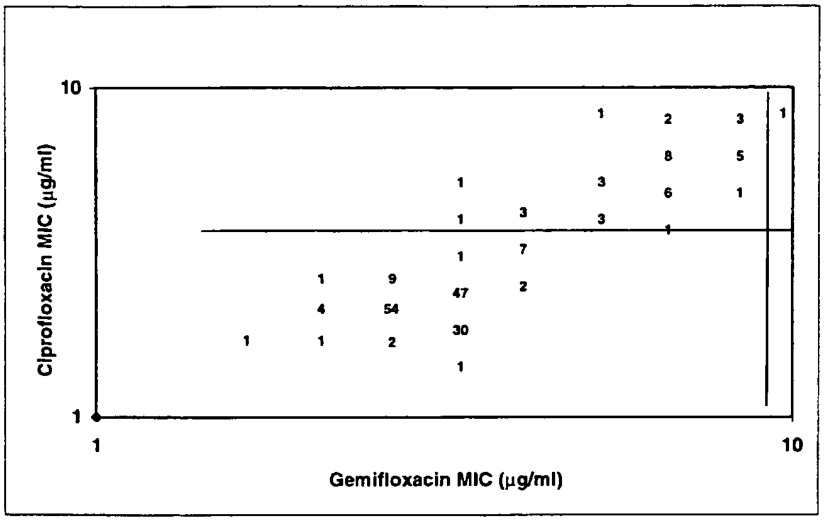
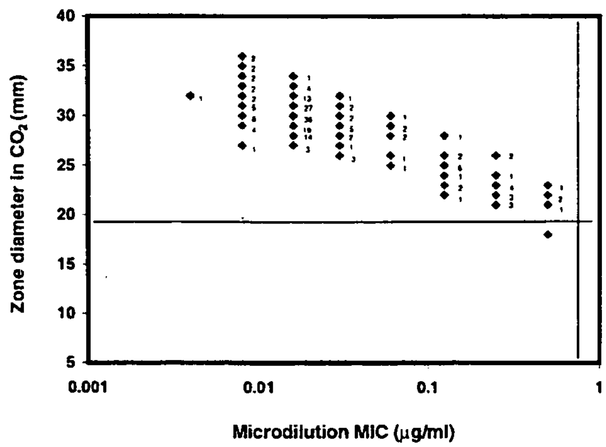

10
1
1
10
Ciprofloxacin MIC (µg/ml)
Gemifloxacin MIC (µg/ml)
1
2
3
1
8
5
1
3
6
1
1
3
3
1
1
7
1
9
47
2
4
54
30
1
1
2
1

Figure 2.


Zone diameter in CO_2 (mm)
40
35
30
25
20
15
10
5
0.001
0.01
0.1
1
Microdilution MIC (µg/ml)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/17900

A. CLASSIFICATION OF SUBJECT MATTER
IPC(7) :A61K 31/47
US CL :514/311, 312
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
U.S. : 514/311, 312

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
NONE

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
CAS-ONLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 4,931,446 A (BERGER-NEEL et al.) 05 June 1990, see the entire document.	1-42
X	US 5,756,506 A (COPELAND et al.) 26 May 1998, see the entire document.	1-42

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search
28 AUGUST 2000

Date of mailing of the international search report
07 SEP 2000

Name and mailing address of the ISA/US
Commissioner of Patents and Trademarks
Box PCT
Washington, D.C. 20231
Facsimile No. (703) 305-3230

Authorized officer
Joyce Bridgers for
KEVIN E. WEDDINGTON
Telephone No. (703) 308-1235

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US00/17900

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☒ Claims Nos.: 43-49
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest
☐ The additional search fees were accompanied by the applicant's protest.
☐ No protest accompanied the payment of additional search fees.